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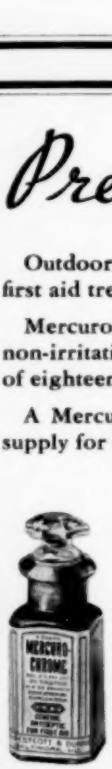

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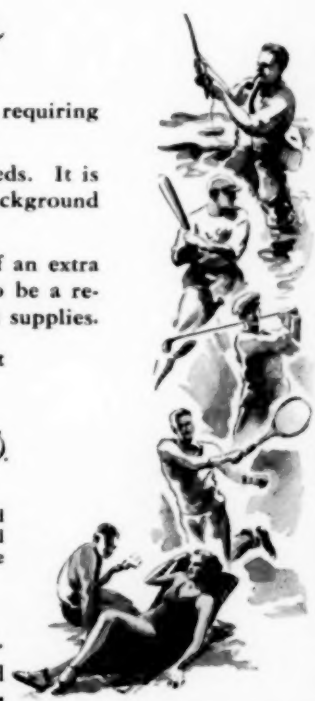

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Quantitative Studies on Pain Threshold after Administration of Various Drugs

*By David I. Macht and Moses B. Macht**

INTRODUCTION

A suitable method for quantitative measurement of the analgesia (as distinguished from local anesthesia) produced by various physical and chemical agents is a crying need of modern experimental physiology, pharmacology and psychology. The old procedure in which psychologists tested pain sensation by the prick of pin or needle applied with varying pressure is obviously neither adequate nor scientifically accurate. The best method for measuring graded pain stimuli in human beings is undoubtedly the electric procedure in which a faradic or induction current is applied to the integument with fine electrodes and the minimum amount of energy required to produce a sensation of pain is determined. Such a method was devised and employed by Macht, Herman and Levy in 1916 in connection with their studies on the comparative analgesia produced by the opium alkaloids and their combinations (1, 2). This method is still regarded as that most satisfactory for quantitative comparison of the effects of well-known, non-toxic drugs on human beings. In this test Macht, Herman and Levy employed a large inductorium calibrated both in the old Kronecker units (3) and also in terms of absolute C. G. S. units. For investigative work on the analgesia of new drugs and chemicals, the clinical toxicity of which has not yet been explored, however, the method mentioned above is unsuitable because of the obvious hazard involved in experimenting on human subjects. Search was therefore made for some other quantitative means of testing analgesia on lower animals. Employing the same method as that used for human beings, the senior author attempted to determine pain thresholds on guinea pigs. This method proved fairly satisfactory in connection with a study of cobra venom analgesia and is described in detail elsewhere (4). Briefly, the procedure was as follows: A small area of skin on the back of the animal is carefully shaved and the threshold of pain for some point in this area is determined with fine electrodes transmitting a graduated faradic current. When the intensity of the faradic current elicits pain, the animal squeals; and the pain threshold thus determined by experienced hands is

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fairly constant. However, several difficulties arise in connection with such a guinea-pig method. In the first place, the animals are inconvenient to handle; in the second place, some of them squeal from fright even when suffering no pain. Furthermore, the pain threshold for the skin on the back of the guinea pig is too high; that is, it requires a powerful stimulation to elicit pain. More recently, the present writers have designed a more suitable method, in which rats are employed instead of guinea pigs, for quantitative study of pain thresholds in animals. It is this new method which is described in the present paper.

EXPERIMENTAL

THE RAT METHOD

In connection with experimental psychological studies on albino rats, it was discovered that the scrotum of adult male rats is very sensitive to faradic stimulation so that the application of fine platinum electrodes to any spot on its surface elicits sharp pain. In this way the threshold of pain for rats can be accurately determined and measured. While an assistant holds the animal gently but firmly, the investigator applies the electrodes at some convenient point on the surface of the scrotum. The faradic current is slowly increased and when the animal receives the first sensation of pain (*i. e.*, when the pain threshold is reached), it utters a characteristic squeal. A normal rat never squeals when merely handled. The pain threshold for any point on the surface of the scrotum is quite constant for at least twelve hours and indeed will be found generally to be the same on the following day unless in the interim the rat has been treated with some pharmacological agent. Figure 1 shows the various areas found most convenient for such determination of pain thresholds. The electrodes may be applied either over the body of the testicles (*A* and *B*) or at their tips (*a* and *b*). Two other convenient spots even more sensitive for the electrodes are over the septum (indicated by the letters *C* and *D*).

Such a "faradic rat method" has been found particularly convenient for measurement of pain thresholds because the scrotum is rich in sensory nerve endings and is much more sensitive to pain stimuli than the skin of the guinea pig. Furthermore the anatomical structure of the scrotum enables the experimenter to distinguish between a general analgesic effect and a purely *local anesthetic* action of a drug. There are two sets of muscles in the scrotum. Some of the muscle fibers in its wall are an extension of the cremasteric muscle; in addition, the so-called dartos tissue itself is composed of muscle fibers. When a *strong* electric stimulus is applied

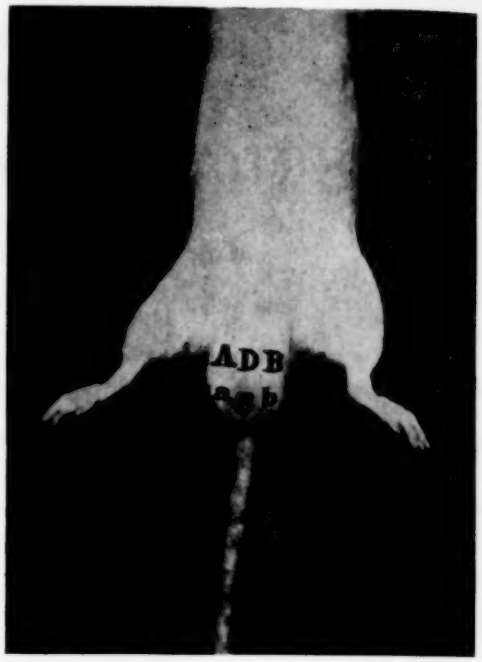

Fig. 1.—Illustration showing site of application of the electrodes in the rat method.

to the surface of the scrotum, pain is elicited not only by direct stimulation of the nerve endings but also by the powerful contractions of the cremasteric and dartos muscle fibers. If a drug is a powerful analgesic, neither the direct stimulation of the sensory nervous apparatus nor the contraction of these muscles and compression of the testes will suffice to elicit pain and cause the rat to squeal. On the other hand, if a local anesthetic is injected intradermally at any point on the scrotum, the failure of a weak electric stimulus to produce a response will indicate local anesthesia, but a stronger stimulus will cause such powerful contraction of the muscles as to elicit pain in the unanesthetized areas and wring a cry from the animal.

Apparatus and Physical Considerations.—In employing the new rat method for determining pain thresholds, the writers made use of the well-known and popular Harvard inductorium. This instrument is constructed of hard rubber and metal. The primary coil is wound with double silk-covered wire, 0.82 mm. in diameter, having a resistance of 0.5 ohm; and its core consists of ninety pieces of shellacked soft iron wire. The secondary coil, which can be moved on two parallel brass rods 22 cm. long, contains 5000 turns of silk-covered wire, 0.2 mm. in diameter. Each end of the secondary wire is fastened to a brass bar screwed to the end of a hard rubber spool. In operating the inductorium, the writers employed a dry cell of 1.6 volts. The intensity of the faradic current was varied, of course, by sliding the secondary toward or away from the primary coil and measuring its distance in centimeters. The effects of drugs on the pain threshold

can be roughly expressed by stating the relative position in centimeters of the secondary to the primary coil. Since this does not convey an adequate idea of the magnitude of the stimuli, when two such thresholds are compared, the authors, with the aid of a distinguished professor of physics, have calibrated their apparatus in absolute physical units and plotted a curve showing the relation between position of the secondary coil to actual voltages for each position. To indicate the vast increase in electromotive force obtained when the secondary is brought closer to the primary coil, it is instructive to dwell briefly on the physical principles of the induction coil.

The induction coil is a device for obtaining induced electromotive forces of high value from an ordinary battery circuit. It consists of a primary coil of, say, n^1 turns of thick copper wire so as to have a small resistance, wound about a central core composed of a bundle of soft iron wires. The coil is connected into the circuit of a battery through the contact point at the end of a set-screw. Outside the primary and thoroughly insulated from it is the secondary coil, consisting of n^2 turns of fine wire, the ends of which come to two insulated posts supporting the discharge electrodes. An iron armature or hammer is provided to make and break the circuit of the primary coil automatically.

If the hammer is held away from the opposite contact point, then touched to this point, and again pulled away quickly, a spark will be seen to pass between the discharge electrodes at the *break* only—*not* at the make. The reason for this is that, on account of the opposing influence at *make* of self-induction in the primary, the magnetic field about the primary rises very gradually to its full strength and hence its lines of force, figuratively speaking, pass into the secondary coil comparatively slowly. At *break*, however, by separating the contact points very quickly, the current in the primary can be made to fall to zero in an exceedingly short time, perhaps not more than 0.0001 second; that is, all its lines of force can be made to pass out of the coil in this time. Hence the *rate* at which lines thread through or cut the secondary is perhaps 1000 times as great at break as at make, and therefore the electromotive force is also something like 1000 times as great.

If a closed circuit be dealt with then the induced electromotive force will depend on the change in the flux through the circuit. If ϕ_1 represents the flux at the beginning and ϕ_2 the flux at the end of the time interval t then

$$E = -\frac{\phi_2 - \phi_1}{t} \text{ (in abvolts)}$$

or

$$E = -\frac{1}{10^8} \cdot \frac{\phi_2 - \phi_1}{t} \text{ (in volts)}$$

where E is the average electromotive force during the time t. The minus sign is used to indicate the direction of the induced electromotive force in accordance with Lenz's law.

If the instantaneous value of the electromotive force is required instead of the average, it is necessary to take but a very small interval of time, dt, at the instant in question and to make the corresponding small change in the flux and then

$$E = -\frac{d\phi}{dt} \text{ (in abvolts)}$$

If ϕ refers to the flux through a coil of one turn, then if there are n turns in the coil, obviously the induced electromotive force will be given

$$E = -n\frac{\phi_2 - \phi_1}{10^8 t} \text{ (in volts)}$$

This brief physical exposition will suffice to show how greatly the voltage of the induced current increases out of proportion to the shifting of the relative positions of the secondary and primary coils in terms of linear distance. The absolute voltages obtained with the inductorium employed in the author's experiments with a dry cell of 1.6 volts in the primary for some of the positions were as follows:

12 cm.—100 volts	6 cm.— 810 volts
10 cm.—105 volts	5 cm.—1223 volts
8 cm.—184 volts	4 cm.—1932 volts
7 cm.—368 volts	3 cm.—2400 volts

Figures were similarly obtained for the voltages at other positions, and a curve was plotted from which the approximate voltage for various pain thresholds could be easily interpolated. The calibrations of the induction coil were made with an R. C. A. cathode ray oscillograph.

It must be borne in mind that such a curve represents only the *peak* voltages of very short duration obtained at the *break* of the interrupting hammer. On superficial thought the high voltages obtained when the secondary is brought close to the primary coil may appear dangerous. From the physiological point of view, however, this is not the case. The fine electrodes are but a few millimeters apart and applied only to the *surface* of the skin. In the second place, the electric shock or stimulation depends not alone on the electromotive force but also on amperage or, in other words, on quantity of current. While the electromotive force may be greatly potentiated in such experiments as are described in this paper, the time of each break of the interrupter is extremely short, about 0.0001 of a second, and the current is so small that no other harmful effect than an instantaneous sensation of pain is produced by application of the electrodes to the surface of the body.

For accurate determinations of pain thresholds, the authors employ a standardized method of procedure. The assistant holds the rat firmly yet gently and the testes are pushed down so as to dis-

tend the scrotum. In warm weather the pendulous testes are fully distended but in cold weather it may be necessary to press the lower part of the abdomen gently to distend the scrotum. The surface to which electrodes are to be applied is first wiped with a wad of cotton dampened with physiological saline. It is most important that the electrodes be always applied to the same points in any one experiment because the threshold of pain, while constant for any two given points, varies with different sensory points on the integument. It is therefore advisable to mark the spot tested in some convenient way.

DRUGS INVESTIGATED

Employing the method described in detail above, the writers tested a large number of drugs for their effect on the threshold of pain in the rat. Among the drugs thus studied were various opiates; a series of coal-tar analgesics such as acetanilid, acetphenetidin, acetyl salicylic acid, phenyl-salicylate, sodium salicylate; alcohol in various dosages; antipyrin, amidopyrin, various barbiturates, urethane, bromides and different alkaloids, some of which were suspected of being sensitizers rather than depressants of the pain threshold. The results obtained with some of these drugs are exhibited in the subjoined tables. Except in case of cobra venom and morphine, the data exhibited therein have been obtained by the use of different rats because the purpose of the present paper is to show primarily the accuracy of the rat-pain-threshold method in testing individual drugs. For comparing the effects of two or more drugs, however, it is desirable, of course, to test them on the same animals on different days. This was done specifically in connection with a comparative study of the analgesia effected by morphine and cobra venom, respectively.

To avoid any ambiguity of expression, it is well to stress the sense in which certain terms are employed in the present exposition. *Pain threshold* is the smallest electromotive force required to produce at a given point sufficient pain to elicit a squeal. When it is stated that the pain threshold is *raised*, it is meant that the secondary has been pushed to within fewer centimeters of the primary coil which, of course, denotes that more energy, more electromotive force in volts is required to elicit pain; hence it indicates analgesia. When the pain threshold is said to be *lowered*, it means that the secondary has been moved some centimeters farther away from the primary coil, and that consequently less energy and electromotive force are required to elicit the first sensation of pain. The animal has therefore become more sensitive.

Effect of Opiates on Threshold of Pain.—The effect of opiates on the threshold of pain is shown in Table I. The opium derivatives examined were morphine sulfate, codeine hydrochloride, heroin, papaverin, pantopon and dilaudid. The table exhibits the dose of the alkaloids injected, and the normal threshold expressed both in centimeters to indicate position of the secondary coil, and in volts. Succeeding columns in the table show any change obtained in pain threshold thirty minutes after, two hours after and, in some cases, four hours after injection of the alkaloids. It will be seen that all the opiates in sufficient doses effected analgesia; *i. e.*, raised the threshold of pain. However, small doses (*e. g.*, 1 mg. of morphine) often had no such effect.

Effect of Other Drugs on Threshold of Pain.—Table II shows the threshold for pain before and after injection of various other analgesics. It will be noted that when *small* doses of *alcohol* (1 cc. of a 5 per cent solution) were injected intraperitoneally, *no analgesia was produced*, as indicated by no change in the pain threshold. Similarly, 2 cc. of a 5 per cent solution produced no alteration. When large doses of alcohol or 10 cc. of a 5 per cent solution, however, were injected into a rat, complete unconsciousness and coma ensued. In such cases, as might have been expected, there was a general anesthesia and the pain threshold, of course, was abolished.

Table I.—Effect of Opiates on Pain Threshold of Rat

Rat No.	Drug Administered	Dose in Mg.	Pain Threshold: Normal Cm.	Normal Volts	15 to 30 Minutes after Dose Cm.	15 to 30 Minutes after Dose Volts	1 to 2 Hours after Dose Cm.	1 to 2 Hours after Dose Volts	Four Hours after Dose Cm.	Four Hours after Dose Volts
1	Morphine sulf.	1	8.0	185	7.6	250	7.8	218	...	...
2	Morphine sulf.	1	6.5	575	6.0	809	6.5	575	...	...
3	Morphine sulf.	1	6.4	630	6.6	530	7.4	280	...	...
4	Morphine sulf.	1.5	7.6	250	6.6	530	7.1	350	7.7	232
5	Morphine sulf.	2.0	7.5	265	6.1	770	7.0	368	7.6	250
6	Morphine sulf.	3.0	7.1	350	5.4	1080	5.8	953	7.7	232
7	Morphine sulf.	4.0	7.0	368	5.6	1018	5.7	983	7.0	368
8	Morphine sulf.	5.0	8.1	180	4.2	1755	0	2852	7.8	218
9	Codeine HCl	2	9.5	125	8.2	176	9.5	125	...	...
10	Codeine HCl	3	8.0	185	7.6	250	8.0	185	...	...
11	Codeine HCl	6	7.0	368	5.0	1223	7.0	368	7.0	368
12	Heroin	3	9.0	100	2.0	2576	2.0	2576	Recovered overnight	...
13	Papaverin HCl	2	7.7	232	7.2	325	7.2	325	7.2	325
14	Papaverin HCl	5	6.8	445	6.3	665	6.8	445	7.6	250
15	Pantopon	2	8.0	185	6.5	575	7.3	300	7.6	250
16	Pantopon	2	8.2	176	7.2	325	7.4	280	8.0	185
17	Dilaudid	0.25	9.0	100	7.0	368	7.0	368	...	...
18	Dilaudid	1.0	7.0	368	4.5	1530	4.8	1330	...	...

Table II.—Effect of Other Drugs on Pain Threshold of Rat

Rat No.	Drug Administered	Dose	Pain Threshold: Normal Cm.	Normal Volts	15 to 30 Minutes after Injection Cm.	15 to 30 Minutes after Injection Volts	One Hour after Injection Cm.	One Hour after Injection Volts	Two Hours after Injection Cm.	Two Hours after Injection Volts
1	Acetanilid	4 mg.	7.8	218	7.8	218	7.0	368	7.5	265
2	Alcohol, 5%	10 cc.	8.0	185	Coma	...	...	...	...	...
3	Alcohol, 5%	2 cc.	8.0	185	8.0	185	8.0	185	...	...
4	Alcohol, 5%	1 cc.	8.5	166	8.5	166	8.5	166	...	...
5	Antipyrin	10 mg.	7.6	250	...	...	7.2	325	7.3	300
6	Atropine sulf.	2 mg.	8.0	185	8.4	170	8.4	170	...	...
7	Barbital Na	10 mg.	8.3	173	...	...	...	...	7.0	368
8	Bromide Na	10 mg.	8.0	185	8.0	185	8.5	166	8.5	166
9	Bromide Na	20 mg.	8.6	162	8.6	162	8.3	173	8.3	173
10	Caffeine	4 mg.	8.1	180	8.1	180	8.4	170	...	...
11	Caffeine	8 mg.	8.2	176	8.2	176	8.3	173	...	...
12	Cocaine HCl	1 mg.	8.0	185	8.1	180	8.1	180	...	...
13	Cocaine HCl	2 mg.	9.5	125	9.8	110	9.8	110	...	...
14	Gynergen	0.2 mg.	8.2	176	8.2	176	8.2	176	8.2	176
15	Phenobarbital Na	5 mg.	7.4	280	...	...	7.8	218	7.7	232
16	Phenobarbital Na	10 mg.	9.0	150	...	...	7.7	232	...	...
17	Quinine sulf.	25 mg.	7.8	218	...	...	7.0	368	7.4	280
18	Salicylate Na	10 mg.	7.2	325	6.8	445	6.8	445	...	...
19	Strychnine nitr.	0.1 mg.	7.3	300	8.0	185	8.2	176	...	...
20	Urethane	40 mg.	7.4	280	...	...	8.0	185	8.2	176

Doses of 5 mg. of the barbiturates, sodium barbital and phenobarbital, effected little or no change in the pain threshold, while larger and toxic doses (10 mg.) definitely lowered it. No analgesia was produced by administration of even 40 mg. of urethane. From the pharmacological standpoint these findings with the barbitals and urethane are of considerable interest because they agree with clinical experience. These drugs are not real analgesics. The rational clinician does not employ the true hypnotic drugs in therapeutic doses for relief of pain but restricts their use to induction of sleep.

Caffeine did not raise the pain threshold; on the contrary, it tended to lower the pain threshold and rendered the rats more excitable. Similar findings were made after injection of small doses of strychnine. Gynergen, recommended by some for relief of migraine, did not raise the threshold of pain. This finding does not argue against its therapeutic efficiency in treatment of migraine but does indicate that the drug exerts an action other than that directly concerned with analgesia.

Of special interest were the data obtained with cocaine. This most potent of local anesthetics is well known to be a delirifacient when given by parenteral or intravenous injection. When cocaine hydrochloride was injected locally between the skin layers of the scrotum so as to produce a wheal, it exerted a powerful and long-enduring local anesthesia, as revealed by electrical stimulation. When the cocaine was injected intraperitoneally or subcutaneously, however, it definitely excited the animal, and the threshold of pain, instead of being raised, was actually lowered.

Threshold of Pain after Administration of Drugs by Stomach.—In Table III are exhibited the effects of various drugs, administered to rats by a special stomach tube. Some of the drugs studied in this connection, such as acetphenetidin and acetyl salicylate, for instance, could not be conveniently injected into the animals. Others, such as morphine and pantopon, are well known to relieve pain whether given by injection or *per os*. It will be noted that while morphine and pantopon administered by stomach tube raised the threshold of pain markedly, indicating the analgesia produced by these narcotics, cobra venom, the new analgesic, introduced in place of morphine for control of pain, effected no alteration of threshold of pain after oral administration. This drug acts only when injected intravenously or parenterally.

Comparison of Cobra Venom and Morphine.—Inasmuch as the use of cobra venom in place of morphine for the relief of pain is rapidly extending, a special study was made of these two medicaments (6, 7, 8, 9, 10, 11, 12). In Table IV are shown the results of a comparative study of morphine and cobra venom. Both drugs were injected intraperitoneally and the dosages employed—1 mg. of morphine and $^1/_2$ a mouse unit of cobra venom,

Table III.—Effect of Various Analgesics Given by Stomach Tube on Pain Threshold of Rat

Rat No.	Drug Administered	Dose Given	Pain Threshold: Normal Cm.	Normal Volts	45 Minutes after Administration Cm.	45 Minutes after Administration Volts	2 Hours after Administration Cm.	2 Hours after Administration Volts	3 Hours after Administration Cm.	3 Hours after Administration Volts
1	Morphine sulf.	6 mg.	8.4	170	6.8	445	7.1	350	8.4	170
2	Pantopon	2 mg.	8.5	166	8.5	166	7.4	280	7.4	280
3	Cobra venom	5 m. u.	8.6	162	8.6	162	8.4	170	8.6	162
4	Acetphenetidin	30 mg.	8.8	155	7.5	265	7.6	250	...	...
5	Acetyl salicylic acid	30 mg.	9.3	135	9.2	140	9.6	120	...	...
6	Phenyl salicylate	30 mg.	7.2	325	7.5	300	7.2	325	7.2	325
7	Amidopyrin	30 mg.	7.6	250	7.5	265	6.0	809	Dead next day	...

Table IV.—Comparison of Cobra Venom and Morphine

Rat No.	Drug Administered	Dose	Pain Threshold: Normal Cm.	Normal Volts	45 Minutes after Injection Cm.	45 Minutes after Injection Volts	2 Hours after Injection Cm.	2 Hours after Injection Volts	4 to 5 Hours after Injection Cm.	4 to 5 Hours after Injection Volts
1	Morphine sulf.	1 mg.	6.0	809	6.1	770	7.0	368	6.0	809
2	Morphine sulf.	1 mg.	6.4	630	6.6	530	7.4	280	6.4	630
3	Morphine sulf.	1 mg.	6.5	575	6.0	809	6.0	809	6.5	575
4	Morphine sulf.	1 mg.	8.0	185	7.2	325	7.2	325	8.0	185
5	Cobra venom	0.5 m. u.	7.0	368	6.6	530	6.7	480	6.5	575
6	Cobra venom	0.5 m. u.	8.3	173	7.5	265	7.2	325	7.2	325
7	Cobra venom	0.5 m. u.	8.3	173	6.8	445	6.3	665	6.2	715
8	Cobra venom	0.5 m. u.	7.2	325	6.5	575	6.1	770	6.0	809
9	Morphine sulf.	3 mg.	7.9	205	4.9	1260	8.0	185	7.9	205
10	Morphine sulf.	3 mg.	7.1	350	5.4	1080	7.9	205	7.2	325
11	Morphine sulf.	3 mg.	8.5	166	6.5	575	8.4	170	8.4	170
12	Morphine sulf.	3 mg.	8.0	185	6.8	445	8.3	173	8.0	185
13	Cobra venom	1 m. u.	8.0	185	7.0	368	4.0	1932	5.0	1223
14	Cobra venom	1.5 m. u.	8.2	176	8.4	170	7.2	325	6.4	630
15	Cobra venom	1.5 m. u.	7.1	350	6.8	445	6.8	575	5.8	953
16	Cobra venom	2 m. u.	8.0	185	6.0	809	5.4	1080	6.4	630
17	Cobra venom	0.5 m. u.	8.0	185	8.1	180	7.5	265	...	...
18	Cobra venom	0.5 m. u.	9.0	150	8.2	176	7.7	232	...	...
19	Cobra venom	0.5 m. u.	8.9	153	7.5	265	7.7	232	...	...
20	Cobra venom	2 m. u.	8.0	185	7.0	368	6.8	445	6.8	445

respectively—roughly corresponded to the relative therapeutic dosage of the two drugs in human subjects. Thus, where $^1/_8$ of a grain or 8 mg. of morphine are administered to a patient, the usual therapeutic dose of cobra venom would be 5 mouse units. The doses employed in the experiments described in this paper, 1 mg. of morphine sulfate and a $^1/_2$ mouse unit of cobra venom, respectively, bear the same ratio as the therapeutic dosage for man of $^1/_8$ of a grain of the former and 5 mouse units of the latter. Similarly 3 mg. of morphine would correspond to 1.5 mouse units of cobra venom. The table reveals that the 1 mg. of morphine produced very little analgesia in some rats and but a mild analgesia in others. On the other hand, $^1/_2$ a mouse unit of cobra venom solution always effected considerable analgesia or increase in pain threshold. While the analgesia produced by morphine is prompt in onset, it generally disappears in a few hours. Cobra venom analgesia, on the contrary, is slow in onset but, once effected, lasts much longer than that of morphine. These experimental findings agree with numerous clinical experiences described elsewhere by the senior author. A partial explanation at least of this striking difference between morphine and cobra venom is supplied in his report of studies on the activity of brain oxydases as influenced by the respective drugs (13).

COMMENT

The present writers describe a new method for quantitative comparison of the analgesia effected by various drugs, which consists of determining the pain threshold for selected points on the scrotum of the adult male rat by means of the faradic stimulus of calibrated induction coil. It was found that the normal pain threshold for any given point on the scrotum of the rat is constant for many hours and can be expressed in absolute physical units. The threshold of pain for selected points on the sensitive scrotum of the normal test animals having been determined, it was also measured for the same points on the same animals after various drugs had been administered by stomach tube. In this way various opium alkaloids were compared with regard to their analgesic action on the rat, and a series of other analgesics and control drugs were examined. The new method has also proved useful in differentiating a purely local anesthetic from a general analgesic action. Thus it was shown that, although cocaine is a powerful local anesthetic, injections of this drug or its salts produced excitement and lowered the pain threshold, *i. e.*, rendered the animals more sensitive. A special comparative study was made of morphine and cobra venom and it was found that both these drugs, when injected in rats, increased the threshold of pain, that is, necessitated an increase of electric stimulation to elicit the first sensation of pain. When the two drugs were given by stomach, a similar effect was produced by morphine but not by cobra venom. A striking difference was noted between the two drugs with regard to time of onset, on the one hand, and duration of their analgesia, on the other. That of morphine was rapid in onset and short in duration. The analgesia of cobra venom,

however, was very slow in onset and of much longer duration. These experimental findings are in complete agreement with numerous clinical data gathered concerning this drug by the senior author.

SUMMARY

1. A new method for quantitative determination of pain threshold in experimental animals has been described.

2. The method consists of applying the faradic current from a standardized induction coil to certain sensitive areas of the scrotum of tame adult male rats and measuring in absolute physical units the minimal energy required to elicit a painful squeal, *i. e.*, the threshold of pain.

3. In complete agreement with clinical experience, a large number of drugs examined in this way were found to produce analgesia in the rat, a circumstance recommending use of this new method in research concerning the analgesia produced by unknown substances.

4. A large number of analgesic drugs (opiates, coal-tar derivatives, alkaloids, etc.), tested in this way, have yielded data running parallel to clinical experience with some medicaments in men.

5. The findings obtained by such a method in studying analgesia produced by morphine and cobra venom agree not only with those derived from studies on guinea pigs and on *normal* human subjects, but also with those obtained from numerous clinical reports.

6. The new method offers a useful and accurate means of detecting analgesic properties of new compounds on which investigative work has not progressed so far as to warrant a clinical trial on human subjects.

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A Pharmacognostical Study of Serenoa Serrulata (Saw Palmetto)*,†

*By B. V. Christensen‡ and R. C. Stokes***

COLLECTION

Information in the literature on the collection of Saw Palmetto berries is questionable and often contradictory. Statements have been made that the time of collection extends from August to January, or even rarely to March (5). Others say that the berries ripen in October and November and may be found until the middle of December (4). Questionable statements regarding the methods of collection are prevalent. For example, the United States Dispensatory (6) states that the fruits are gathered when fully mature, partially dried artificially and packed in barrels, and that, to the contents of each barrel, a small amount of alcohol is added as a preservative.

In order to learn more exactly the time of collection of the berries and to obtain accurate information concerning the methods of collection, several trips were made by the writers to localities in Florida from which the commercial supply of Saw Palmetto berries

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is at present obtained. These trips included visits to the east coastal region of Florida, from Daytona Beach south to New Smyrna, to Coronado Beach and to Canaveral Island (Cape Canaveral). By visiting these localities, by conversing with experienced collectors of Saw Palmetto berries and by observing the types of apparatus used in collecting and drying the berries, much valuable information was obtained. From this information, the writers present the following method of collection of Sabal berries. In their opinion, it is the one most commonly employed at present.

Time of Collection.—The berries are collected when they are full grown and ripe. Consequently, the time of collection varies, within limits, each year according to climatic variations. As a general rule, the time of collection begins as early as September 1, and continues until January 20. Berries gathered during mid-season (October 15 to December 20) appear to be of a better quality than those collected at other times. The early- and late-gathered berries seem to be deficient in oil and syrupy exudate. This deficiency of oil and syrupy exudate causes the berries to become hard and quite brittle after they are dried.

The fruits appear to be more abundant in alternate years. Sometimes, however, there may be several consecutive years in which few berries are produced. Following this scarcity, the fruits may be abundantly produced for several consecutive years. There appears to be no satisfactory explanation for this inconsistency of fruit production.

Procedure of Collection.—Experienced white collectors are employed for the collection of the fruits. The fruiting stalk, containing the ripe fruits, is cut with pruning shears. The stalk is then shaken over an ordinary bean hamper, the berries falling into the hamper (see Fig. 1). After the hampers are filled with berries, they are carried to a shed where the berries are transferred to trays for subsequent sun-drying.

Drying Procedure.—The berries are placed in lath-slatted drying trays. These trays are about three and one-half inches deep, three feet long and two feet wide. They are usually about half-filled with berries and then placed on inclined wooden benches or racks. These racks are so constructed that the trays rest on them at an angle of about 30 degrees.

In this position, the berries are allowed to dry in the sun for a period of six to eight weeks. During this drying process, care must be exercised to prevent the berries from becoming too moist from exposure to excessive rainfall. One or two rains aid the process by washing off the "syrupy" fluid which exudes from the berries during drying. More than one or two rains are injurious, resulting in molding and decaying. In order to protect the berries from too much moisture, tarpaulins are spread over the trays during the rains.

Fig. 1.—Fruiting Stalk of Sabal with Berries.

As the drying process continues, berries that have become thoroughly dried are removed from the trays by hand picking and fresh, undried berries added. The partially dried berries are placed at the bottom of the trays in order to give them greater protection from rains.

After the fruits have become thoroughly dried, they are removed from the trays and garbled (hand sorted). Those which are not well filled and whose surfaces are not creased or wrinkled are discarded and usually fed to animals. The good berries are placed in wooden or tin containers until ready for shipment.

The berries are not placed in brine or alcohol during the period of storing and shipping. In fact, no preservative is added. The fruits are shipped either in the fresh, ripe state, in the semi-moist state or in the dried condition.

The above method of collection is the one used today by conscientious collectors. However, there are many unscrupulous collectors of Saw Palmetto berries who make no attempt to follow this procedure. They collect berries which are still green; they have few, if any, trays or racks; and they make no attempt to garble the berries, but ship all fruits collected.

It appears that many firms who buy and use Saw Palmetto berries are not aware of differences in quality of the fruit. As a result, they are at the mercy of unscrupulous collectors.

HISTOLOGY

A review of the literature shows that little study has been devoted to the histology of the fruit and seed of Saw Palmetto. Also, microscopical studies of the powdered fruit and seed appear to be meager.

John M. Maisch (2) in 1883 published J. Moeller's description of the fruit and seed of this drug. This description is mostly macroscopic. His drawing of the pericarp is fairly accurate, but the few cells of the endosperm of the seed which are illustrated do not clearly represent this region.

Henry H. Rusby (5) in 1895 studied the structure of the fruit and seed of Saw Palmetto. His description is entirely macroscopic. This publication includes no drawings.

H. Kraemer (1) in 1910 made the most complete microscopical study of the fruit and seed that has been made up to the present. His description and drawing of the three outer rows of the fruit (which he calls epicarp) are accurate, but his drawing of the cells of the adjoining layers (which he terms sarcocarp) does not accurately represent this region; he describes these cells as being nearly isodiametric. Actually, these cells are of irregular shapes and sizes. His description of the cells of the inner layer of sarcocarp is much too brief, and he does not show them in his drawing. He mentions that sieve, cambium and spiral vessels constitute the fibrovascular bundles. No cambium cells were seen by the writers in the fibrovascular bundle. Kraemer reports the absence of rosette crystals of calcium oxalate; yet these were occasionally found by the writers to be present.

There are also discrepancies in Kraemer's drawing of the seed. The testa is not composed of one layer of thin-walled, tangentially elongated cells, but is made up of several layers of thin-walled, irregularly shaped cells. The cells of the endosperm are shown in his drawing as being radially elongated; in reality, those cells are of various shapes. Also, the canals in the walls of the endosperm cells appear to be occluded pores, more or less flared at their distal ends, rather than rounded, slightly tapering penetrations of protoplasm as they seem to be represented in Kraemer's illustration. His description of the seed is much too brief. He does not describe the seed coats or the cells of the perisperm.

The description in the United States Dispensatory (7) of 1937 is incomplete and without detail. The description of powdered Sabal in the National Formulary VI (3) is incomplete in that it does not include all of the elements found in the powdered fruit and seed. No mention is made of the elements comprising the epicarp, testa, tegmen, perisperm or of the fibrovascular bundles.

These few scattered descriptions comprise the available studies of the histology of the fruit, seed and powdered drug. From these, it is apparent that a complete, accurate study of the histology of the fruit, seed and powdered drug is needed. Therefore, this present investigation was undertaken.

Source of Material.—The fruits and seeds used in this study were obtained from plants growing on Canaveral Island or Cape Canaveral, Florida (see collection of fruits).

Method of Treating Material for Microscopical Examination.—Special methods of treatment had to be devised in order to render the fruit and seed suitable for sectioning. The seed had first to be removed from the pericarp, and then each treated separately and sectioned separately. This removal of the seed was accomplished by making a transverse incision around the middle portion of the fruit, removing one-half of the pericarp and then taking out the seed.

The seeds were softened by boiling in water for 24 hours and then treated with a 25 per cent solution of hydrofluoric acid for a week, after which they were thoroughly washed in water and then placed in a mixture of equal parts of 95 per cent alcohol and glycerin for a week or longer. The softened seeds were embedded in paraffin and transverse sections made of them by the use of a rotary microtome. The sections were cut approximately 15μ in thickness.

The fruit, with seed removed, was steamed on a water bath for two hours. The pericarp was then cut into longitudinal strips about one and one-half cm. long and one-half cm. wide. These strips were embedded in paraffin and transverse sections, 15μ

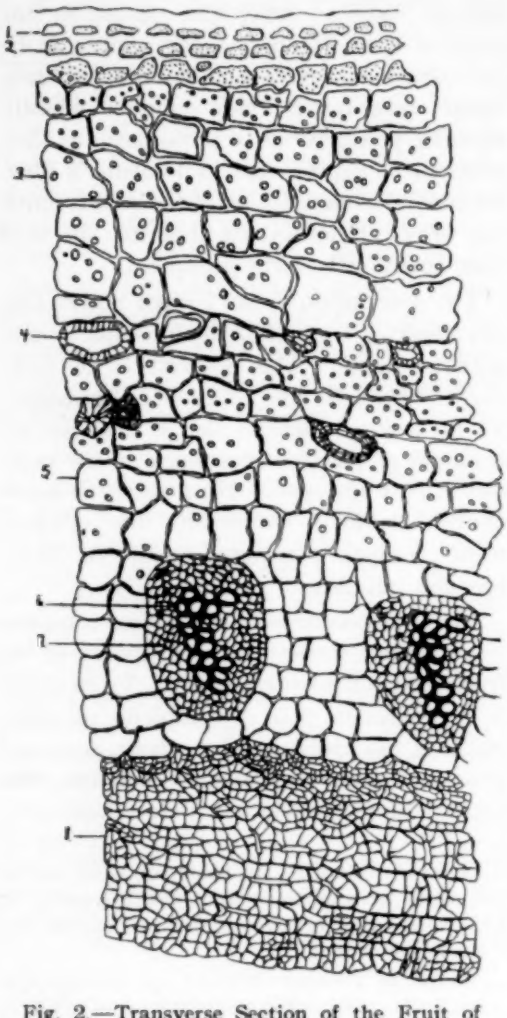

Fig. 2.—Transverse Section of the Fruit of *Serenoa Serrulata*.

in thickness, made of them by the use of a rotary microtome.

Transverse sections of embedded pericarp and seed were macerated in xylol for ten minutes in order to dissolve the paraffin. They were then treated for ten minutes with each of the following: xylol, absolute alcohol, 95 per cent alcohol, 75 per cent alcohol, 50 per cent alcohol and then placed in water. Various staining reagents were used for the purpose of more clearly differentiating the tissues. Those used were: safranin, Delafield's haematoxylin, phloroglucin hydrochloride, Bismarck brown, methylene blue, chlorozinc-iodine, alcanna tincture, sudan-glycerin, acetic acid, hydrochloric acid, 50 per cent sulfuric acid, 90 per cent alcohol, iodine water, chloral hydrate, 50 per cent glycerin, 5 per cent potassium hydroxide solution and concentrated potassium hydroxide solution.

After staining, the sections were examined under a compound microscope using low power magnification (100×) and high power magnification (440×). All drawings were made from high power magnification.

Method Used for Powdering the Material.—The fruits, with seeds removed, were placed in an electric oven at a temperature of 100° C. for twelve hours in order to remove any moisture present. They were then ground in a drug mill to a number 40 powder.

The seeds, after being removed from the pericarp, were thoroughly washed in water to remove the "syrupy" exudate adhering to them. They were then dried by placing in an electric oven at 100° C. for twelve hours. The seeds were ground to a number 40 powder by the use of a drug mill.

These powders were then placed in tightly closed bottles until ready for examination.

Description of Transverse Section of the Fruit.—(See Fig. 2.) The *epicarp* is composed of three regions: (1) *epidermis*, (2) *subepidermis* and (3) *inner epicarp region*. The **epidermis** (1) is composed of one layer of cells, the walls of which are strongly cutinized. The thick cuticle is of a straw color, and varies from 8 to 9μ in thickness. These epidermal cells are small, varying from 10 to 40μ in width and from 20 to 44μ in length. The shapes of their lumina are irregular, some being almost square, others rectangular to tangentially elongated and some in the form of an undulate triangle. The middle lamella of the cell wall is difficult to distinguish. These cells are filled with a yellowish to reddish brown amorphous substance.

The **subepidermis** (2) is composed of one to two layers of cells similar in appearance to those of the epidermis, but differing from them in not having their walls cutinized and being averagely of a larger size. The walls of these cells are of a cellulose nature. The width of these cells varies from 15 to 24μ; their length varies from 30 to 48μ.

The **inner epicarp region** (3) is composed of from 5 to 10 layers of cells containing a reddish amorphous material, which gives this region a distinct red appearance. The cells of this region show many variations in shape, but the majority of them are tangentially elongated; others are almost square, to round, to triangular in shape. These cells are much larger than those of the other two regions of the epicarp; their walls are also thickened. The length of these cells varies from 100 to 310μ, their width from 33 to 190μ. The majority of these cells contain one to several yellowish colored oil globules.

Interspersed among the cells of this region are occasional round to elongated, lignified[1] stone cells, some of which contain an orange colored amorphous material in their lumina. The size of these stone cells varies from 73 to 105μ in length and from 45 to 65μ in width. The thickness of their walls varies from 5 to 15μ. Striations and pores are not evident in these cells.

[1] The term "lignified" as used in this and the following descriptions signifies a positive color reaction (some shade of pink to red) with acidified phloroglucin hydrochloride reagent.

The **mesocarp** (5) is the middle region of the pericarp. It is composed of 25 to 30 layers of cells, only some of which are shown in Fig. 2. These cells are also irregular in shape, some being tangentially elongated, others radially elongated, while those nearer the endocarp are usually approximately isodiametric in shape. These inner layers of the mesocarp, in which the fibrovascular bundles are located, are composed of smaller cells than those making up the outer layers of this region. The length of the cells of the outer layers of the mesocarp varies from 55 to 263μ; their width varies from 37 to 165μ. The cells of the outer region contain yellowish colored oil globules, but the cells of the inner layers appear to be devoid of them. The mesocarp cells do not contain reddish colored amorphous material such as is found in the epicarp cells.

Interspersed among the cells of the outer layers of the mesocarp are **lignified stone cells** (4). These stone cells appear to be of three types. Those of the first type have no evident pore canals or striations; those of the second type show no striations but have evident pore canals, some of which are branched; those of the third type have walls much thicker than those of the first and second types. These cells of the third type show both striations and pore canals, some of the latter being branched. The length of the stone cells varies from 33 to 189μ; their width varies from 20 to 137μ. The diameter of the lumen varies from 4 to 60μ. The thickness of their walls varies from 5 to 36μ. The shape of these stone cells varies from square, to round, to oval, to elongated.

The **fibrovascular bundles** (6 and 7) are located in the inner region of the mesocarp. These bundles are of the concentric type—phloem surrounding xylem. No cambium cells are evident. The xylem (6) occupies the central portion of the bundle, and is composed mostly of lignified, spiral vessels; few, if any, wood fibers are present. The phloem (7) surrounds the xylem, and is composed of sieve tubes, companion cells, phloem parenchyma and a few scattered bast fibers. Longitudinal and oblique views of fibrovascular bundles are occasionally seen; that is, some of the bundles appear to be running obliquely and at right angles to other bundles.

Intercellular air spaces are found throughout the mesocarp region. Most of these are small, some are fairly large. Occasionally, rosettes of calcium oxalate are seen in the cells of this region.

The **endocarp** (8) is composed entirely of strongly lignified stone cells having thick, porous walls. This region is composed of 7 to 12 layers of tightly fitting cells. These cells vary in shape from square, to round, to tangentially elongated; some are radially elongated. Their length varies from 15 to 46μ, their width from 15 to 45μ. All of these cells show evident pore canals, but few striations are evident. The walls of these stone cells are very thick (12 to 17μ); their lumina are small (5 to 10μ in width).

Description of Transverse Section of the Seed.—(See Fig. 3.) 1. **Testa.** This region is composed of 6 to 14 layers of irregularly shaped, tightly fitting cells, most of which are tangentially elongated. The walls of these cells are thick (4 to 12μ) and contain numerous pores. The walls of the outermost layers appear to be lignified. Many of these cells are filled with a reddish brown amorphous substance. The length of these cells varies from 11 to 225μ; their width varies from 8 to 80μ. These variations in the size of the cells were not evident in all of the sections examined. In some sections, this region appeared to be composed of cells of more or less uniform size; while in other sections extreme variations in sizes were noted. In sections where variations of sizes occurred, the smaller cells were located

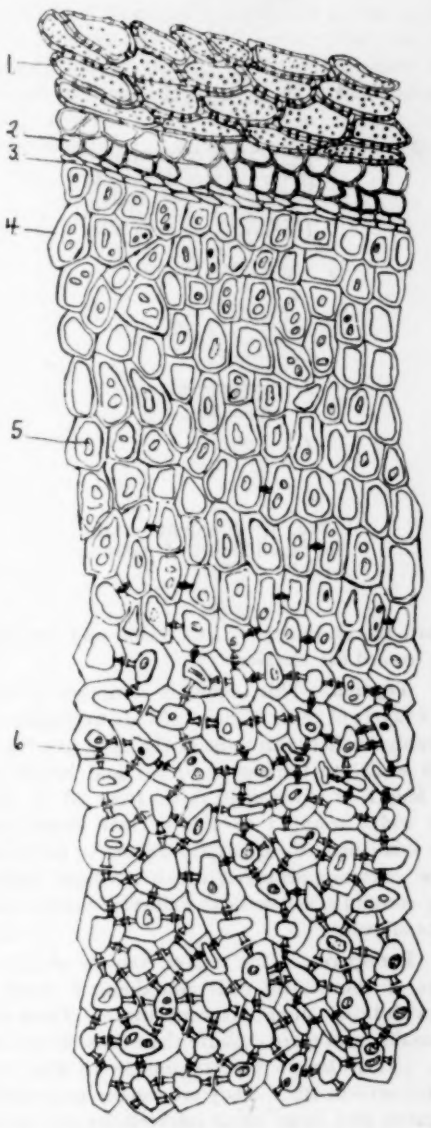

Fig. 3.—Transverse Section of the Seed of *Serenoa Serrulata*.

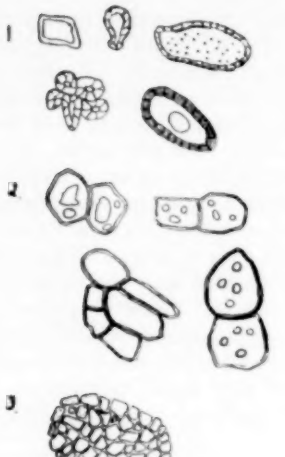

Fig. 4.—Elements of the Powdered Fruit of *Serenoa Serrulata*.

toward the periphery of this region and the larger cells toward the interior.

2. **Tegmen.** This region is composed of from 2 to 4 layers of cells. These cells are averagely considerably smaller than those of the testa (see Fig. 3). Their walls are thinner (2 to 4μ) and non-pitted. The length of these cells varies from 20 to 160μ, their width from 11 to 48μ. Their shapes vary from triangular, to square, to oblong to polygonal. These cells are arranged in an irregular fashion. They contain a yellowish to yellowish brown amorphous substance.

3. **Perisperm.** This region consists of 2 to 3 layers of thin-walled cells containing a straw to yellowish brown amorphous substance. These cells are smaller and more delicate than those of the tegmen. Their length varies from 18 to 32μ, their width from 5 to 12μ. They are usually tangentially elongated and have small intercellular air spaces. Sometimes the cells comprising this region are crushed and torn, and may be difficult to distinguish.

4. **Endosperm.** This region constitutes the largest portion of the seed. It is composed of many layers (40 to 50) of cells with thick, colorless walls. The cells comprising the outer layers of this region are somewhat radially elongated, but there is a gradual change in the shapes of the cells and a gradual increase in wall thickness as the center of the seed is approached. The cells comprising the middle portion of the endosperm are more elongated radially than the cells of the outer or inner layers. The innermost portion of the endosperm is composed of polygonal-shaped cells; their walls are also colorless and very thick. These cells of the central portion are characterized by striking canals in their walls (see 6 of Fig. 3). The distal ends of these pores are more or less flared and lie opposite similar pores of adjacent cells. The appearance of these canals would seem to indicate the probable presence of plasmodesma. However, close examination failed to reveal any such protoplasmic strands. These canals appeared to be occluded pores in the cell wall, rather than penetrations of protoplasm as apparently indicated by Kraemer's drawing (1). The cells of the endosperm vary in length from 20 to 148μ; their width varies from 20 to 45μ.

Many of these cells contain irregularly shaped, yellowish bodies; these are aleurone grains. An occasional oil globule was seen in a few of these cells.

Description of the Elements of the Powdered Fruit (*Pericarp*).—(See Fig. 4.) 1. **Stone Cells.** These cells are numerous and are found throughout the powder. Some of these occur as isolated cells, but the majority of them are found in groups. These stone cells appear to be of three types. Those of the first type are slightly lignified, have no evident pore canals and are nonstriated; those of the second type show no striations but have evident pore canals, some of which are branched; the cells of the third type show both striations and pore canals, some of the latter being branched. Stone cells of this third type are more numerous and their walls are thicker than those of the first and second types. Stone cells of the first type appear to be less strongly lignified than those of the second and third types.

Stone cells of the powdered fruit vary much in shape (see 1 of Fig. 4). They may be round, or oval, or ellipsoidal, or square, or spatulate or tabular. The size of these cells varies from 20 to 137μ in width and from 33 to 189μ in length. The thickness of their walls varies from 5 to 36μ, and the diameter of their lumina varies from 4 to 60μ. In the majority of stone cells the lumen appears to be empty, but occasionally an oil globule is found in the cavity.

2. **Cells of the Mesocarp.** These cells have somewhat thickened, colorless walls. The majority of the mesocarp cells contain a pale yellow to straw colored amorphous content. Many of them contain one to several oil globules. In shape, these cells vary from round, to square, to polygonal to oblong.

Their size varies from 37 to 165μ in width and from 55 to 363μ in length.

3. **Epidermal Cells as Seen in Surface View.** These cells are irregular in shape, varying from triangular, to square, to round, to oval, to polygonal to elongated. They have comparatively thick walls, but the middle lamella is difficult to differentiate. These cells contain a yellowish to reddish brown amorphous content. In size, they vary from 10 to 40μ in width and from 20 to 44μ in length.

4. **Fragment of Epicarp Showing Cells of Epidermis and Subepidermis.** The epidermis is composed of one layer of cells, the walls of which are heavily cutinized. The cuticle of these cells is of a straw color and varies from 8 to 9μ in thickness. These epidermal cells are small, varying from 10 to 40μ in width and from 20 to 44μ in length. The shapes of their lumina are irregular, as noted above, and they are filled with an amorphous content.

The subepidermis is composed of two layers of cells similar in appearance to those of the epidermis, but differing from them in not having their walls cutinized and in being averagely of a larger size. The width of these cells varies from 15 to 24μ, and their length varies from 30 to 48μ.

5. **Sclerenchyma Fibers.** These fibers are found in groups. They are usually lignified and their walls show many pore canals. Fibers are only occasionally found in the powder and then usually as broken fragments. They are very much elongated and have thick walls with narrow lumina. The thickness of their walls varies from 13 to 16μ; the widths of their lumina vary from 8 to 14μ. The lengths of these fibers are from 240 to 434μ; their widths are from 36 to 40μ.

6. **Cells of Inner Epicarp.** These are rather large cells with thick, colorless walls. Some of these cells have walls which are finely porous. Cells of the inner epicarp are filled with a reddish to reddish brown amorphous material. In shape, these cells vary from triangular, to round to elongated. Their length varies from 100 to 310μ; their width varies from 33 to 190μ. Many of these cells contain one to several yellowish colored oil globules.

7. **Fragment of Spiral Trachea.** Vessels are rarely found in the powdered fruit. They are slightly lignified with moderately thick walls. Only vessels of the spiral type are found in the powder. The tracheal fragment (as shown in 7 of Fig. 4) is 20μ in width and 76μ in length.

8. **Oil Globules.** These are found scattered throughout the powder; many of them are free and not located in cells. Their color varies from straw to pale yellow, and their size varies from 4 to 56μ in diameter.

The noteworthy elements of the powdered pericarp are the stone cells, the mesocarp cells and the cells of the inner epicarp.

Description of the Elements of the Powdered Seed.—(See Fig. 5.) 1. **Cells of Testa.** These cells are irregularly shaped, varying from square, to oblong, to triangular to elongated. The walls of these cells are comparatively thick (4 to 12μ) and usually contain numerous pores. Many of the cells have lignified walls, some showing heavy lignification, others showing only slight lignification, while a few show no lignification. There is a wide variation in the size of these cells; their length varies from 11 to 225μ, their width from 8 to 80μ. The widths of their lumina vary from 4 to 56μ. An occasional oil globule may be seen in the lumina of a few of these cells.

These cells of the outer seed coat are numerous, and are frequently found in groups scattered throughout the powder. Occasionally, isolated cells are seen. Many of the testa cells are filled with a reddish brown amorphous substance.

2. **Cells of Tegmen.** These cells are not as frequently seen as those of the testa. They are also averagely smaller than the cells of the outer seed coat and their walls are thinner (2 to 4μ) and nonpitted. However, a few of these tegmen cells appear reticulated in surface view (see 2 of Fig. 5).

Fig. 5.—Elements of the powdered Seed of *Serenoa Serrulata*.

These cells are non-lignified, and they contain a yellowish to yellowish brown amorphous material. In shape, they vary from triangular, to square, to polygonal to oblong. They vary in length from 20 to 160μ and in width from 12 to 48μ. These cells are arranged in an irregular fashion as shown in 2 of Fig. 5.

3. **Cells of Perisperm.** These are delicate, small, thin-walled cells. Their length varies from 18 to 32μ and their width varies from 5 to 12μ. Their cell walls are only 1 to 2μ in thickness. In shape, these cells vary from oval to elongated. Very few of them are seen in the powdered seed, and usually they are found as broken fragments. The perisperm cells contain a yellowish to yellowish brown amorphous material.

4. **Cells of Endosperm.** These cells are more numerous than any of the other elements of the powdered seed. They are of various shapes and sizes. In shape, they may be square, or oval, or polygonal or elongated; in size, they vary from 20 to 45μ in width and from 20 to 148μ in length. Some of these cells have walls uniformly thickened; others have irregularly thickened walls; while still others show markedly thickened walls with large, characteristic pore canals (see 4 of Fig. 5). These cells have been previously described under the heading of the transverse section of the seed. These endosperm cells have thick walls, the thickness varying from 8 to 20μ. The width of their lumina varies from 14 to 30μ. Many of these cells contain yellowish colored, irregularly shaped aleurone grains; an occasional oil globule may be seen in some of these cells.

The most outstanding elements of the powdered seed are the testa cells and the endosperm cells.

SUMMARY AND CONCLUSIONS

1. The authors present a method of commercial collection of Saw Palmetto berries which, in their opinion, is the one most commonly employed at present. This method is based on knowledge obtained by visiting the actual localities where much of the commercial supply of Saw Palmetto berries is collected, by conversing with experienced collectors and by observing the types of apparatus used in collecting and drying the berries.

2. An accurate study of the histology of the fruit and seed (both unground and ground) is presented. Drawings and descriptions of transverse sections of the fruit and seed and of the powdered fruit and seed are included in this study.

3. This histological study of Sabal shows that certain changes and additions should be made in the present monograph on Powdered Sabal of the National Formulary VI. The authors, therefore, recommend that this monograph be reworded and changed to read as follows:

"Powdered Sabal: Yellowish brown, fragments of inner epicarp, the cells containing a reddish to reddish brown amorphous material and yellowish colored oil globules; yellowish fragments of mesocarp, the cells having somewhat thickened colorless walls and containing a pale yellow to straw colored amorphous material and one to several oil globules; whitish fragments of endosperm numerous, the cell walls of which may or may not be uniformly thickened and usually showing large, characteristic pore canals; numerous stone cells, colorless to straw colored, varying in shape from tabular, to oval, to polygonal to elongated, from 0.020 mm. to 0.189 mm. in length, with walls from 0.005 mm. to 0.036 mm. in thickness, many of these cells showing numerous simple or branching pores and polarizing light with a distinct cross; occasional fragments of sclerenchyma fibers, having thickened walls and narrow lumina; fragments of spiral vessels only rarely present; straw to pale yellowish oil globules scattered throughout the powder, many of which are free and not located in cells; fragments of testa numerous, the cells usually containing a reddish to reddish brown amorphous substance; occasional fragments of tegmen, the cells having thin, non-pitted walls and containing a yellowish brown amorphous substance."

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Chemical Studies on a Physiologically Active Substance in Passiflora Incarnata*

By G. H. Ruggy and C. S. Smith

Ott (1) in 1898, DeNito (2) in 1931, and Fellows and Smith (3) in 1938 established the presence of a physiologically active substance in Passiflora. Ott and Fellows and Smith investigated the species *incarnata* while DeNito investigated five other species. The principal interest in the United States is in the species *incarnata* since it is this species that has been and still is in use in medicine. Its use today depends upon its supposed sedative action. Ott and DeNito reported depressant effects on the central nervous system after the administration of aqueous solutions of alcoholic extracts of the crude drug. Fellows and Smith were unable to demonstrate any such action, but they and DeNito reported the presence of some substance which produced marked lowering of the blood pressure in dogs.

DeNito was of the opinion that this substance is an alkaloid or a mixture of alkaloids but Fellows and Smith failed to confirm this observation.

The present investigation was undertaken with a view to establishing more clearly, if possible, the chemical nature of the physiologically active substance in *Passiflora incarnata*.

EXPERIMENTAL

Procedure.—Weighed portions of the crushed stems and leaves of *Passiflora incarnata* were extracted with three volumes of 85 per cent alcohol on a boiling water-bath. The extract was filtered and the alcohol was removed from the filtrate by vacuum distillation. The residue was dissolved in water and an excess of basic lead acetate was added. The resulting lead precipitate was removed by filtration. The filtrate after being freed from lead with mercuric sulfide was reduced to a syrup *in vacuo*. This syrup was mixed with plaster of Paris and allowed to set. The plaster was powdered in a mortar, then dried for six hours in a vacuum oven at 60° C. The powdered plaster was next extracted with absolute alcohol for 24 hours in a shaker and centrifuged. The alcoholic extract prepared in this manner was treated with an excess of 20 per cent solution of mercuric chloride in absolute alcohol and a heavy white precipitate obtained. The precipitate was filtered by suction and washed with absolute alcohol and anhydrous ether. The mercury precipitate thus obtained was found to contain the physiologically active ingredient of Passiflora.

In attempting to purify the mercury precipitate the crude substance was dissolved in water and the solution filtered to remove a slight cloudiness. This aqueous solution was divided into three parts, *A*, *B* and *C*, and subjected to the following procedures.

Portion *A* was poured into a large (50×) excess of a mixture of equal parts of ether and acetone. The resulting precipitate was collected by decantation and centrifugation.

The water was removed from Portion *B* by distillation with immiscible solvents using butyl alcohol. The residue insoluble in butyl alcohol was collected.

The mercury was removed from Portion *C* as mercury sulfide and the mercury-free solution subjected to the plaster of Paris procedure previously described and again precipitated from absolute alcohol with mercuric chloride.

The three residues, *A*, *B* and *C*, resulting from these procedures were alike in appearance and behavior. They were analyzed for carbon, hydrogen, nitrogen and mercury (see Table I). Residue *A* was too small to provide samples for combustion analysis.

Carbon and hydrogen were determined by the combustion method using reduced copper, metallic silver and gold foil to remove nitrogen, chloride and mercury, respectively.

Mercury was determined by reduction with formaldehyde, solution of the metallic mercury in nitric acid and titration of the mercurous nitrate with standard potassium thiocyanate using ferric alum as an indicator.

Nitrogen was determined by the Kjeldahl method.

Table I.—Results of Analyses of Residues

Residue	C, %	H, %	N, %	Hg, %
A	...	...	2.64	30.05
	...	...	2.69	31.00
B	23.61	3.98	2.67	29.98
	24.01	4.11	2.71	30.16
C	25.31	4.21	2.69	31.40
	25.24	4.29	2.71	30.98

The uniformity of these results was taken as evidence that the mercury derivative was probably a pure compound.

The extraction of the crude drug was such a time-consuming procedure that a short-cut was sought. Accordingly, a sample of fluid extract of Passiflora was tested to see whether the fluidextract contained any appreciable amount of the active substance. Such was found to be the case and four liters of fluidextract, supplied through the courtesy of Eli Lilly and Company, were subjected to the procedure outlined at the beginning of this discussion. The mercury precipitate obtained was treated as was Portion *C* described above and a yield of approxi-

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mately 5 Gm. resulted. This preparation was used for the remainder of the chemical work and for the pharmacological work which is to be described in a later paper.

The mercury precipitate thus prepared was an amorphous brownish white powder. It was very hygroscopic and slowly turned brown on exposure to light and air. It therefore had to be kept in a light-proof vacuum desiccator.

The mercury derivative was very soluble in water, slightly soluble in 95 per cent alcohol and insoluble in ether, chloroform, benzene and other organic solvents. It has thus far resisted all attempts at crystallization.

Qualitative analysis revealed the presence of mercury, chloride and nitrogen but no phosphorus or sulfur. Quantitative analysis showed the following composition:

C—23.44%	N— 2.74%
H— 4.44%	Hg—31.35%
O—25.23% (by difference)	Cl—12.80%

Carbon, hydrogen, nitrogen and mercury were determined by the methods already described. Chloride was determined gravimetrically. Oxygen was determined by difference.

Molecular weight determinations were not successful. On the basis of elementary analysis and assuming that only one atom of nitrogen is present in each molecule, the molecular weight should be 511. On the same basis, an empirical formula derived from the elementary analysis would be $C_{10}H_{22}O_3N.HgCl_2$. This compound has a molecular weight of 548. A similar series of calculations could be made based on the assumption that only one atom of mercury is present. However, it will be seen in Table II that the relation based on nitrogen is quite good while that based on mercury is poor. Inherent difficulties in the volumetric determination of mercury may be responsible for low results in the table, or it is possible that the combination of the active substance with mercuric chloride is a loose one and that in the purification procedure some mercuric chloride was lost.

Table II.—Chemical Composition

Element	% Composition	% Atomic Wt.	Relation Based on N	Relation Based on Hg
C	23.44	1.952	10.0	12.51
H	4.44	4.404	22.6	28.23
O	25.23	1.577	8.0	10.1
N	2.74	.195	1.0	1.25
Hg	31.35	.156	.8	1.00
Cl	12.80	.361	1.85	2.31

The specific rotation was determined using aqueous solutions of the mercury derivative. Samples varying in amounts from 0.4 Gm. to 0.6 Gm. per 100 cc. of solution were used. The specific rotation at 20° C. varied from −18.01 to −18.21 degrees of angular rotation. The average was −18.16 degrees.

An aqueous solution of the mercury precipitate was used for qualitative tests with the following reagents:

Molisch reagent—greenish color in the sulfuric acid and a definite purple ring at the interface.
Fehling's reagent—slight greenish precipitate.
Bromine water (saturated)—decolorized.
Alkaline potassium permanganate—decolorized.
50 per cent sodium hydroxide—heavy gelatinous precipitate.

Benedict's, Fuchsin aldehyde, Tollen's, Wagner's, Nessler's and Mayer's reagents, and Hinsburg's, Simon's and Riminis tests all gave negative reactions.

It has been difficult to obtain the mercury compound in sufficient quantity to permit the preparation of derivatives. Attempts have been made to acetylate and to brominate the compound but have been unsuccessful. Oxidation with potassium permanganate has been attempted and certain substances have been obtained by extraction of the oxidized mixture with immiscible solvents. These products are crystalline but have been isolated in such very small quantities (1–2 mg.) as to preclude identification.

It is not possible at this time to draw any definite conclusions as to the nature of the active principle of *Passiflora incarnata*. It is reasonably certain that it is not an alkaloid. It may possibly be a glycoside as indicated by the positive Molisch reaction. However, this too, seems unlikely since no evidence of the presence of glycosides could be obtained by the emulsin procedure of Bourquelot (4) run on the semicrude extract following the lead precipitation.

SUMMARY

A physiologically active substance has been isolated from *Passiflora incarnata*, in the form of a mercury derivative. An empirical formula is proposed but no data are available as yet which will permit the structural identification of the compound.

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NOTICE

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Crystalline Tannin from the Bark of Acer Spicatum*

By J. L. Powers† and E. L. Cataline‡

The bark of *Acer spicatum* has been reported as an adulterant of, or substitute for, *Viburnum opulus* (1), but heretofore no information concerning its chemical constituents has been presented. *Acer spicatum* is not mentioned by Wehmer (2), and the only reference to it is that by St. John (3), which relates to the recognition of it in galenical preparations by means of a color test. In connection with a chemical study of *Viburnum opulus* it seemed advisable to investigate *Acer spicatum* bark in order to determine if the chemical constituents of the two drugs were at all similar.

From a preliminary chemical examination of the bark of *Acer spicatum*[1] by a general method (4), it soon became apparent that the main constituent was a seemingly amorphous tanning, which is by no means true of *Viburnum opulus* (5, 6). It was finally found upon further purification of the apparently amorphous tannin that a crystalline compound, $C_{20}H_{20}O_{13}.2H_2O$, could be isolated from it. This compound exhibited certain characteristics of a true tannin, *e. g.*, precipitation of alkaloids and gelatin, and the tanning of goldbeater's skin. The goldbeater's skin test, originally devised by Atkinson and Hazleton (7) and later modified by Price (8), is considered by Nierenstein (9) to be the only true criterion of a natural organic tannin.

This tannin appeared to be identical in physical and chemical properties with crystalline acer-tannin isolated from the leaves of *Acer ginuala* by Perkin and Uyeda (10). Like acer-tannin, it hydrolyzed to form one molecule of aceritol and two molecules of gallic acid. Freudenberg and Rogers (11) have recently shown that aceritol and polygalitol from *Polygala senega* are identical. Accordingly, the aceritol from *Acer spicatum* tannin was identified by comparison with polygalitol isolated from *Polygala senega* root. An octoacetyl derivative, $C_{36}H_{32}O_{21}$, was also prepared which was similar in physical and chemical properties to the corresponding acer-tannin derivative prepared by Perkin and Uyeda (10). Moreover the tannin methylated by means of diazomethane yielded a crystalline hexamethyl derivative, $C_{26}H_{32}O_{13}$, a compound which Perkin and Uyeda were unable to obtain in a crystalline condition. Hydrolysis of the methylated tannin by the method of Fischer and Freudenberg (12), while not conclusive, indicated the presence of trimethyl gallic acid and the absence of dimethyl gallic acid in the hydrolysate.

EXPERIMENTAL

Isolation of Crystalline Tannin.—Seven Kg. of granulated *Acer spicatum* bark[2] were exhausted by extraction with 95% alcohol in a Lloyd extraction apparatus. The alcoholic extract was concentrated to a syrup-like consistence which weighed 1.6 Kg. To this extract were added with vigorous stirring 4.5 l. of water. The mixture was filtered, and the filtrate after standing 12 hours had deposited 55 Gm. of a red amorphous material. Upon longer standing, an additional 190 Gm. of this material was obtained. The 245 Gm. were dissolved in hot water, and the solution partially decolorized with charcoal. After several recrystallizations from water, 44 Gm. of colorless crystals melting at 165–166° with decomposition were obtained. $[\alpha]_D^{27°} = +17.5°$ (2.5% in acetone).

Analysis: Calcd. for $C_{20}H_{20}O_{13}.2H_2O$: C, 47.62; H, 4.76.
Found: C, 47.67, 47.47; H, 4.92, 5.01.

The tannin, air-dried at 100°, did not lose water of crystallization. However, when dried at 135–140°, it lost 7.05% of its weight. Calcd. for $C_{20}H_{20}O_{13}.2H_2O$: H_2O, 7.14%.

Analysis: Calcd. for $C_{20}H_{20}O_{13}$: C, 51.28; H, 4.28.
Found: C, 51.02, 51.38; H, 4.49, 4.51.

The intensity of color on goldbeater's skin (8) produced by a 0.2% aqueous solution of the tannin from *Acer spicatum* was about the same as that exhibited when a 0.2% aqueous solution of gall-tannin was used. Likewise, the precipitation effects upon aqueous salts of brucine, cinchonidine and strychnine, and upon a 1% aqueous solution of

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[1] This investigation was made possible by a grant from the Faculty Research Fund of the University of Michigan.

[2] Purchased from the S. B. Penick Company.

gelatin in 10% sodium chloride were almost identical.

Hydrolysis of the Tannin.—Since attempts to hydrolyze the tannin by the method of Fischer and Freudenberg (13) did not yield quantitative results, the following method was finally devised. A mixture of 3 Gm. of tannin and 150 cc. of 5% sulfuric acid was heated on the steam-bath during 24 hours, cooled, partially neutralized with sodium carbonate (8 Gm.), concentrated to a volume of 50 cc. and placed in a refrigerator. After 48 hours the gallic acid which had precipitated was removed by filtration. The gallic acid in the filtrate was extracted by shaking with three 15-cc. portions of ethyl acetate. The extracts were combined, washed with water, shaken with sodium chloride and evaporated. The combined gallic acid residues were dried in a vacuum desiccator over phosphorus pentoxide. Yield, 2.19 Gm. Calculated for $C_{20}H_{20}O_{13}.2H_2O$, 2.24 Gm. After one recrystallization from water the gallic acid melted at 238–240° with decomposition. A mixture of gallic acid (Eastman) showed no depression in melting point.

The aqueous solutions were combined, neutralized to Congo Red paper with sodium carbonate and evaporated to dryness. The residue was extracted with 150 cc. of absolute alcohol, and the extract evaporated to dryness. Yield, 0.78 Gm. Calculated for $C_{20}H_{20}O_{13}.2H_2O$, 0.98 Gm. Recrystallized from alcohol the aceritol melted at 143–144°. When mixed with polygalitol isolated from *Polygala senega* by the method of Shinoda, Sato and Sato (14), there was no depression in melting point.

Acetylation of the Tannin.—Two Gm. of tannin from *Acer spicatum* were heated during 2 hours under a reflux condenser with a mixture of 10 cc. of anhydrous pyridine and 8 cc. of acetic anhydride. The solution was then cooled to room temperature and poured into 50 cc. of a 5% aqueous solution of sulfuric acid to which ice had been added. The acetylated tannin was extracted from this by means of ether, the etheral solution was washed several times with water, dried with anhydrous sodium sulfate and the ether removed by distillation. The residue, recrystallized once from methyl alcohol, formed ball-like masses of microcrystalline needles melting at 155–156°. Yield, 2.5 Gm. Calculated, 3.17 Gm.

Analysis: Calcd. for $C_{36}H_{36}O_{21}$: C, 53.73; H, 4.47; CH_3CO—(acetyl group), 42.77.
Found: C, 53.58, 53.86; H, 4.47, 4.61; CH_3CO—, 43.14, 43.30.

Methylation of the Tannin.—Two Gm. of the tannin were dissolved in 15 cc. of absolute methyl alcohol. To this solution was added an excess of an ether solution of diazomethane prepared from phenylnitrosourethane by the method of von Pechmann (15), and the mixture was placed in a refrigerator. After 12 hours, the white crystalline methyl derivative was removed by filtration. Yield, 1.26 Gm. Calculated, 2.21 Gm. The melting point after recrystallization from a mixture of methyl alcohol and ether was 172–174°, with preliminary softening.

Analysis:[3] Calcd. for $C_{26}H_{32}O_{13}$: C, 56.52; H, 5.79; —OCH_3, 33.69.
Found: C, 56.15, 56.47; H, 6.07, 5.94; —OCH_3, 34.01, 33.91.

Hydrolysis of the methylated tannin with 10% methyl alcoholic sodium hydroxide (12) yielded a small amount of a white substance which was insoluble in a mixture of equal parts of carbon tetrachloride and chloroform. Presumably this substance was trimethylgallic acid.[4] However, it was impossible to obtain quantitative results from the hydrolysis of the methylated tannin.

SUMMARY AND CONCLUSIONS

1. A crystalline tannin has been isolated from the bark of *Acer spicatum*.

2. Both the tannin and the products of hydrolysis are similar in chemical and physical properties to acer-tannin and its products of hydrolysis.

3. A crystalline octoacetyl derivative of the tannin has been prepared. This derivative is similar in chemical and physical properties to the octoacetyl derivative of acer-tannin obtained from *Acer ginuala* leaves.

4. A crystalline hexamethyl derivative of the tannin from *Acer spicatum* has been prepared, but a crystalline methyl derivative of acer-tannin obtained from *Acer ginuala* has not been described.

5. Since diazomethane seldom, if ever, attacks alcoholic hydroxyl groups, the preparation of a hexamethyl derivative indicates strongly that the original tannin must possess a structure including six phenolic hydroxyl groups. This can be explained only upon the assumption that the original tannin molecule is composed of aceritol to which are attached two separate galloyl nuclei, and not one meta- or para-digalloyl nucleus.

6. It is believed that evidence has been presented to indicate strongly that the tannin from *Acer spicatum* bark and the tannin obtained from *Acer ginuala* leaves are identical.

[3] Analyses of the methylated tannin were done in the Laboratory of Microchemistry of Dr. Carl Tiedcke, 366 Fifth Avenue, New York.
[4] *Cf.* ref. 12.

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2-Alkylmercurithiopyridine-5-Carboxylic Acids*,†

Preparation of, and Stability of Their Solutions

By Lewis A. Walter and Russel J. Fosbinder

INTRODUCTION

In 1922 Kharasch (1) and Vieth (2) described some mercury compounds prepared by reacting a mercurial of the type R Hg X with a mercapto acid of the type R′ S H where R′ is a group containing an alkyl or aryl carboxylic or sulfonic acid. The resulting compounds, having the general formula R Hg S R′ Ac could be dissolved in solutions of sodium bicarbonate or alkali metal hydroxides. Later Kharasch (3) applied the same general method to the preparation of alkylmercurithio compounds more stable in character and suitable for medicinal application.

The preparation, antiseptic efficiency and toxicity of a series of alkyl derivatives were reported by Waldo (4) in 1931. One derivative, ethylmercurithiosalicylate, has since found widespread use as an external antiseptic.

Alkylmercurimercapto compounds, in general, when dissolved in aqueous solutions in the form of their alkali metal salts acquire medicinally undesirable properties, presumably as a result of the splitting-off of the alkylmercuri radical at the sulfur linkage, due to the oxidation of the mercapto compound or residue to the disulfide form. Although oxidation proceeds slowly at room temperature, in the presence of minute concentrations of certain metal ions, such as copper, iron and manganese acting as metallic catalysts, the normal oxidation is accelerated, resulting in marked deterioration over a period of several days. Kharasch (5) was able to stabilize aqueous solutions of these compounds by the addition of small amounts of aliphatic amines and diamines, the latter type of compound being most effective.

This paper describes alkylmercuri derivatives of 2-mercaptopyridine-5-carboxylic acid. Stability tests carried out at room temperature and at 60° C. on aqueous solutions of sodium 2-ethylmercurithiopyridine-5-carboxylate indicate that compounds of this type are substantially resistant to oxidation, even in the presence of metal ion catalysts.

EXPERIMENTAL

PREPARATION OF 2-ALKYLMERCURITHIOPYRIDINE-5-CARBOXYLIC ACIDS

An excess of alkylmercuric chloride, prepared by the Grignard reaction (6), was added to an alkaline alcoholic solution of 2-thiopyridine-5-carboxylic acid. After refluxing for 1 hour the solution was concentrated to one-half volume, diluted with water and filtered. The mercury compound was then precipitated with acetic acid and recrystallized from alcohol.

The compounds were analyzed for mercury by oxidizing with fuming nitric and sulfuric acids, then with permanganate, and titrating with potassium thiocyanate.

STABILITY OF SOLUTIONS

Oxidation Tests.—Stock solutions of the mercury compounds were prepared by dissolving 2 Gm. of the alkylmercurithiopyridine carboxylic acid in the theoretical quantity of 1% sodium hydroxide solu-

* Presented before the Division of Medicinal Chemistry, American Chemical Society, Milwaukee, September 8, 1938.

† From the Research Laboratories of The Maltbie Chemical Company.

Table I.—Melting Points and Mercury Contents of Alkylmercuri Derivatives of 2-Mercaptopyridine-5-Carboxylic Acid

Derivative	M. P., ° C.	Formula	Per Cent Mercury Calcd.	Found
2-Ethylmercuri	250 decomp.	$C_8H_9O_2NSHg$	52.22	52.14
2-*n*-Propylmercuri	210 decomp.	$C_9H_{11}O_2NSHg$	50.45	50.28
2-*n*-Butylmercuri	190 decomp.	$C_{10}H_{13}O_2NSHg$	48.69	48.42

tion and diluting to 1 liter. Unless otherwise noted, a solution of this concentration was always employed in making up the test solution, the final concentration of the mercury compound being 1 Gm. in 1000 cc. Double distilled water prepared by a method similar to that described in the N. F. VI (7) was employed as the solvent.

Aliquot portions of the test solution were sealed in clean ampuls and either heated at 60° C. or permitted to stand at room temperature (ca. 25°) for the observation period. At the proper time the ampuls were opened and appropriate samples, generally 5 cc., withdrawn for analysis. Initially a series of experiments was carried out in which oxygen was bubbled through the solutions contained in the ampuls, but no appreciable acceleration of deterioration was observed as compared to those ampuls containing air above the liquid. Consequently all of the data reported in this paper were obtained on solutions having an atmosphere of air above them during the test period.

A quantitative measure of the deterioration, due to oxidation, of the mercapto compounds was obtained by titrating samples, acidified with hydrochloric acid, with *N*/200 iodine. In preliminary experiments it was found that exactly one equivalent of iodine was necessary to oxidize one molecule of alkylmercurithiopyridine compound to the disulfide acid.

Results.—The observations reported in Tables II and III show the apparent stability of sodium 2-ethylmercurithiopyridine-5-carboxylate in aqueous solution, and in the presence of metal ion catalysts usually effective in promoting the oxidation of mercapto compounds.

From the extent of deterioration of the mercuri compound in the absence of a catalyst and in the presence of ethylene diamine which was added as an additional stabilizer it seems justifiable to assume that a large part of the small loss is due to some factor other than catalytic oxidation. It is to be noted from Table III that the concentration of copper ion was increased to 1:50,000. This concentration of Cu ion was employed in order to obtain a maximal effect for copper. From the observations of Elliott (8) on the catalytic oxidation of cysteine and thioglycolic acid it may be assumed, however, that in so far as copper is concerned a 1:1,000,000 concentration is sufficient for a catalytic maximum.

For the purpose of comparing the stability of 2-ethylmercurithiopyridine-5-carboxylic acid with a related compound, ethylmercurithiosalicylate was prepared according to the procedure described by Waldo (4). A solution of the sodium ethylmercurithiosalicylate was made up by dissolving 1 Gm. of the free acid in the calculated amount of 1% sodium hydroxide solution, adding 1.4 Gm. of sodium borate, 1.0 Gm. of sodium chloride, sufficient copper sulfate to yield a copper ion concentration of 1:1,000,000 and diluting to 1 liter. Aliquot parts of the solution were sealed in glass ampuls, one sample allowed to stand at room temperature for 163 hours, a second sample heated at 60° C. for a period of 237 hours, while a third sample, to which 1% of ethylene diamine had been added, was similarly treated. On titrating the test solutions at the end of the experiment the extent of oxidation was found to be 51, 96 and 0.0 per cent, respectively. From Table I it is seen that the thiopyridine salt, under

Table II.—Stability of a 1:1000 Solution of 2-Ethylmercurithiopyridine-5-Carboxylic Acid (in Form of Sodium Salt) at 60° C.

Buffered solutions contain: sodium borate 0.14%; sodium chloride 0.10%

Solution	Catalyst	Per Cent Oxidized after Heating 237 Hours	405 Hours	573 Hours
Unbuffered p_H 11.0	None	3.82	6.88	7.63
Buffered p_H 8.8	None	4.96	6.88	6.50
Buffered p_H 8.8	Mn 1×10^{-6}	2.67	5.35	3.43
Buffered p_H 8.8	Fe 1×10^{-6}	0.00	2.29	0.00
Buffered p_H 8.8	Cu 1×10^{-6}	4.96	9.92	12.20
Buffered p_H 8.8 1% $C_2H_4(NH_2)_2$	Cu 1×10^{-6}	4.58	8.41	9.94

Table III.—Stability of a 1:1000 Solution of 2-Ethylmercurithiopyridine-5-Carboxylic Acid (Sodium Salt) at Room Temperature

Solution	Catalyst	Per Cent Oxidized After 13 Weeks	39 Weeks	70 Weeks	96 Weeks
Unbuffered p_H 11.0	None	0.37	1.88	3.39	3.01
*Unbuffered p_H 11.0	None	0.00	1.56	3.84	5.76
Unbuffered p_H 11.0	Cu 1:50,000	0.00	3.76	11.65	9.77
Buffered p_H 8.8	None	0.00	1.50	3.75	0.37
Tincture	None	0.73	0.00	1.84	2.95

* Stored in an amber glass bottle.

the same experimental conditions, deteriorated only 4.9% as compared to 96% for the thiosalicylate compound.

Inhibition of Catalytic Oxidation by 2-Ethylmercurithiopyridine-5-Carboxylic Acid.—Since 2-ethylmercurithiopyridine-5-carboxylic acid resisted substantial catalytic oxidation for long periods of time at room and elevated temperatures it might be assumed that this compound forms a coördination complex, catalytically inert in character, with copper. If a catalytically inactive complex is formed between copper salts or ions and the mercapto pyridine compound then the latter should inhibit the catalytic oxidation of a mercapto compound susceptible to such action. To test this hypothesis a series of solutions, the compositions of which are indicated below, was prepared, placed in vials and heated at 60° C. At hourly intervals 5-cc. samples were withdrawn, chilled immediately and titrated with $N/200$ iodine solution.

Solution *A*—100 cc. contain:

Sodium ethylmercurithiosalicylate	0.05 Gm.
Sodium borate	0.14 Gm.
Sodium chloride	0.10 Gm.
Cu	0.10 mg.

Solution *B*—100 cc. contain:

Sodium ethylmercurithiosalicylate	0.05 Gm.
Sodium 2-ethylmercurithiopyridine-5-carboxylate	0.05 Gm.
Cu	0.10 mg.
Sodium borate	0.14 Gm.
Sodium chloride	0.10 Gm.

Solution *C*—100 cc. contain:

Sodium ethylmercurithiosalicylate	0.05 Gm.
Sodium 2-ethylmercurithiopyridine-5-carboxylate	0.05 Gm.
Sodium borate	0.14 Gm.
Sodium chloride	0.10 Gm.

Table IV.—Inhibition of Oxidation of Sodium Ethylmercurithiosalicylate by Sodium 2-Ethylmercurithiopyridine-5-Carboxylate. t = 60° C.

Hours Heated	Per Cent Oxidation		
	Solution *A*	Solution *B*	Solution *C*
1	28.7	2.1	0.6
2	33.4	0.0	0.0
3	41.6	0.0	0.0
4	46.7	0.9	0.0
5	51.4	4.8	0.0

From the results it is seen, at least for the interval of time indicated, that 2-ethylmercurithiopyridine-5-carboxylic acid effectively prevented the catalytic oxidation of ethylmercurithiosalicylate, and therefore the copper ions must have been bound by complex formation with the thiopyridine compound. It should be observed that the complex formed in this instance is not of the type formed by other sulfhydryl compounds such as cysteine (9), glutathione, etc., with metal ion oxidation catalysts.

Sodium 2-thiopyridine-5-carboxylate does not form a catalytically inactive complex with metal ion catalysts for in the presence of Cu 1:1,000,000 a 1:1000 solution of this salt, heated at 60° C. for 1 hour, was oxidized 20%, and when heated at 60° C. for 237 hours over 96% was oxidized. In the absence of added copper this compound, similarly treated for 237 hours, deteriorated 85% and was therefore susceptible to autocatalytic action in the presence of atmospheric oxygen.

The addition of a number of pyridine compounds to solutions of sodium ethylmercurithiosalicylate containing Cu 1:1,000,000 exerted no action in inhibiting the oxidation of the compound. In these experiments pyridine, 2-aminopyridine, 2-pyridone and 2-mercaptopyridine were studied.

SUMMARY

1. The preparation of some new organic mercurials has been described.

2. Oxidation tests carried out on one of the compounds indicate that they are substantially resistant to catalytic oxidation.

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Note on the U. S. P. XI (Supplement II) Monograph on Cyclopropane*

By G. H. W. Lucas and V. E. Henderson

This Department has been studying the standards of purity for Cyclopropane for a year or more but did not make rapid progress until the Research Division of the Ohio Chemical Company kindly provided us with small cylinders of a specially purified cyclopropane, of methyl acetylene, propylene and allene. Our thanks are due to them for making this study possible.

In the preparation of cyclopropane several unsaturated gases might be formed, and indeed in various commercial samples as much as 2% or over of unsaturated com-

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pounds have been found, as will be reported below. The unsaturated compound usually considered to be present is propylene. The boiling point of cyclopropane according to the International Critical Tables 1927, is −34.5° C. at 760 mm. Ashdown (1) gives −32.89° C. The boiling point of propylene is −47.67° C. and might be removed by fractional distillation. A sample of cyclopropane prepared in this laboratory when fractionated by expert low-temperature distillation contained mere traces of propylene. The anesthetic value of propylene has been adequately studied (2, 3, 4). Secondly allene, $CH_2{=}CH{=}CH_2$, might occur as an impurity. Beilstein gives its boiling point as −32° C. and it might have been present in the sample of cyclopropane referred to, as it would not have been detected in the fractionation. Its anesthetic action has been studied by Ferguson (5). The third compound which certain organic chemists expected would occur is methyl acetylene, $CH_3{\equiv}C{-}CH$, which has a boiling point of −23.5° C. The method of Illosvay van Nagy Illosva (6) for the detection of acetylene yields with methyl acetylene a yellowish precipitate. When a large volume of commercial cyclopropane was slowly bubbled through this reagent no trace of yellow precipitate occurred. Henderson (7) has recently studied the anesthetic properties of this gas. It is further possible that hexenes might be produced, but owing to their high boiling point these would probably be removed by fractionation. We are therefore chiefly concerned with allene and propylene.

EXPERIMENTAL

Definition and Assay.—The definition of cyclopropane in the U. S. P. XI, Second Supplement, states that "Cyclopropane contains not less than 99% $CH_2{-}CH_2{-}CH_2$," *i. e.*, 99% cyclopropane. Yet in the method of assay, namely, absorption of the gas from the cylinder in sulfuric acid, propylene and allene, as well as the cyclopropane, will dissolve if they are present.

According to this method of assay "the residual gas . . . . does not exceed 1% of the volume of the original gas taken for assay." That is, according to the assay one per cent of the original gas may consist of other impurities than unsaturated hydrocarbons, namely air, carbon dioxide and carbon monoxide, all of which would not be absorbed by sulfuric acid. Furthermore, the method does not say whether the gas for analysis is to be taken from the liquid or the gaseous phase, which is likely to contain more air than the liquid one. Our samples have been drawn from the gaseous phase and in some cases commercial samples, as shown below, contain as much as 1% of gas unabsorbable in sulfuric acid; in one or two cases slightly more was present. It is possible that all these gases would have qualified if the sample for analysis had been drawn from the liquid phase.

In addition to the assay with sulfuric acid which will allow the presence of 1% of unabsorbable gas, the monograph sets a limit for the amount of unsaturated impurity, allene and propylene, and as will be shown below this may amount to another 1% or more. Consequently the requirement that the gas must be 99% cyclopropane seems hard to justify.

Tests for Identity and Impurity.—The one test to which we wish to refer is that for unsaturated hydrocarbons, *i. e.*, propylene and allene. A liter of cyclopropane is passed through a column 12–14 cm. deep and containing 50 cc. of $N/100$ potassium permanganate in 15 minutes. The orifice from which the bubbles arise is defined and the solution kept during the process at 3° C. ±2. In order to pass this test "the volume of hundredth-normal potassium permanganate reduced by the gas is not more than 10 cc. (propylene, allene and other unsaturated hydrocarbons)."

We soon found much difficulty in obtaining satisfactory duplicate results in applying this method, using either samples of commercial gas or percentage mixtures made up from propylene and practically pure cyclopropane furnished us by the Ohio Chemical Company. The first experiments were performed by aspirating a liter of gas through the permanganate by means of a suction pump. The irregularities in results were attributed to the difficulty of performing this test in exactly 15 minutes. It was also observed that the bubbles were not uniform in size. Consequently a flask was filled with a saturated solution of calcium chloride which had been saturated with cyclopropane, and this solution was displaced by the gas to be examined; in turn the gas was displaced from the flask by the same saturated solution which entered the flask from a funnel fixed above to give a constant pressure head. The level of the funnel was maintained almost constant by an inverted Mariotte flask, the oblique orifice of which just entered the fluid in the funnel.

With the addition of this method of securing a more uniform rate of bubbling, all conditions were strictly adhered to in the experiments reported in detail, though approximately the same quantitative results were obtained in many other experiments where the bubbling was not so closely controlled, and are not reported in detail. Constancy of rate of bubbling becomes particularly important when the amount of the impurity is large, but even more important is the constancy of size of bubbles which one fails to obtain in such a crude apparatus. During the bubbling operation one can observe the for-

mation of large and small bubbles. Large bubbles appear to break at times when the tip of the bubbler is above a certain point in the cylinder containing the permanganate. Moving the tip very little results in giving bubbles of different sizes, with consequent different titration of permanganate. A spiral of glass about the bubbling tube was tried in order to break up the bubbles, but even with this small and large bubbles were present and results were not uniform.

According to specifications, commercial gases must contain 99% of cyclopropane. If no gas unabsorbable in sulfuric acid is present, then the 1% may be propylene. In order to discover if the disappearance of 10 cc. of permanganate corresponded to the presence of 1% of propylene in a liter of gas, samples of gas prepared by adding 10 cc. of propylene to 990 cc. of special cyclopropane were bubbled through permanganate. The special cyclopropane used up about 1.8 cc. of permanganate. In Table I, which gives the results of these analyses, this amount has been deducted.

Table I.—Cubic Centimeters of $N/100$ Potassium Permanganate Used up by 1 Liter 11% Propylene in Cyclopropane

Date	Time in Minutes	Cc. of $N/100$ $KMnO_4$ Soln.
Feb. 2	14	11.9
Feb. 2	15	9.3
Feb. 2	16	12.9
Feb. 6	15	9.4
Feb. 7	15	9.1
Feb. 10	18	10.9
Feb. 27	16	11.9
Feb. 27	16	10.3
Feb. 27	15½	14.1
Mar. 2	15¼	9.2
Mar. 2	16½	11.2
Mar. 2	16	11.9
		Av. 10.9

When a 2% propylene in cyclopropane was passed, the figures were in all cases less than was to be expected from those found for a 1% propylene, for example, 17.2, 16.4, 12.3, 15.8.

We would conclude from our experiments that the reduction of 10 cc. of permanganate would correspond approximately to 10 cc. of propylene in a liter, *i. e.*, 1%, and that the method becomes quantitatively less accurate as the content of propylene increases beyond 1%. To demonstrate that bubbling a liter of 2% propylene through permanganate did not remove all the propylene, a liter flask was fitted with a 2-hole stopper. Through one hole a short glass tube was passed, the outer end holding a small balloon. Through the other hole a capillary tube was passed until it almost touched the bottom of the flask. Fifty cubic centimeters of permanganate were placed in the flask and 20 cc. of propylene were passed in, requiring about 2 minutes, the flask being well agitated during this interval. By this means the propylene was brought into intimate contact with the permanganate and subsequent titration showed that on two such trials 25.7 and 25.5 cc. of permanganate disappeared, as compared with 17.2, 16.4, etc., as cited above.

When a liter of 1% allene in cyclopropane was tested, so much of the permanganate was reduced that reproducible results could not be attained. Consequently, a mixture containing 0.1% in the purified Ohio cyclopropane was used. The average result of several runs was 7.9 cc. of permanganate reduced (spread from 7.7 to 8.1). A 0.2% mixture reduced only 14.6 cc. (instead of 15.8 cc.), while a 0.58% mixture reduced only 25.4 cc. and 1.3% only 30.2 cc.

Here, again, and even more strikingly than with propylene, the permanganate method does not give quantitative results and is only approximately quantitative when 10 cc. or less of permanganate are reduced.

It is also obvious that the standard set, namely, the reduction of not more than 10 cc. of permanganate, would not allow the presence in cyclopropane of more than a little over 0.1% of allene, while it would allow about 1% of propylene. Turning to the results of applying this method to commercial samples, it is very obvious that very low percentages of allene in a gas would condemn it, although it might contain 99.8% cyclopropane.

Owing to a suggestion from the Imperial Chemical Company in Great Britain prior to the publication of the Supplement, we had been testing Wijs solution as a method of estimating the amount of unsaturated impurity. We had used various methods of absorbing in the Wijs solution and finally found that the use of 25 cc. of Wijs solution in the small bubbler described in *Industrial and Engineering Chemistry* (9) gave very satisfactory results when a liter of gas was passed in half an hour.

When a liter of the specially purified Ohio gas was passed through Wijs solution, 0.4 cc. of $N/10$ thiosulfate solution was the equivalent of the Wijs solution attacked. This calculated in terms of propylene is 0.05%. The permanganate method on the same gas showed that 1.8 cc. of $N/100$ were reduced. This again means a small amount of propylene but seems to be substantially higher than the amount revealed by the Wijs method. Again, this amount has been deducted from the results with prepared mixtures.

Using a 1% mixture of propylene in cyclopropane, approximately theoretical results were obtained, the average result being that 8.8 cc. of thiosulfate were the equivalent of the Wijs solution disappearing. In terms of propylene this would be 10.47 cc. instead of the 10 cc. actually employed.

When several analyses of 1% of allene in cyclopropane were made, the results were always higher than calculated for the breaking of one double bond; namely, Wijs solution equivalent to 13.9 cc. of thiosulfate disappeared, which is equivalent to 16.2 cc. calculated as propylene instead of 10. For the saturation of both double bonds the calculation of 10 cc. of allene should equal 20 cc. of propylene.

A twenty-five cc. sample of Wijs solution may be employed to measure mixtures up to 2% of propylene and will give approximately quantitative results.

From the figures reported it is evident that Wijs solution will give a more accurate measure of the amount of propylene present than the permanganate method and this is true of allene as well.

Our very early tests on commercial gases with the Wijs solution convinced us that the unsaturated hydrocarbons varied considerably. Tests with the permanganate method, both by aspirating the gases through permanganate or forcing them through as indicated above, showed similar variation. Five analyses made on a tank purchased March 1, 1940, showed that 13.2, 13.9, 11.9, 10.4, 12.4 cc. of $N/100$ permanganate were reduced. When unsaturated impurities are present in small amounts, less variation was obtained, another tank showing 1.8, 1.9, 2.0, 2.0, on the four occasions tested. The amount of gas unabsorbable in sulfuric acid was estimated in some samples and again some variation was apparent. Table II gives some results obtained by the permanganate and Wijs methods, together with the amount of unabsorbable gas in some commercial samples tested.

Table II.—Absorbable Gas and Results with the $KMnO_4$ and Wijs Methods

Manufacturer	Per Cent of Gas Unabsorbable by Sulfuric Acid	Cc. of $N/100$ $KMnO_4$ Reduced by 1 L. of Gas	Wijs Method, Cc. of $N/10$ $Na_2S_2O_3$ Soln. per L. of Gas	Unsaturated Hydrocarbons, % Calculated as Propylene	
				$KMnO_4$ Method	Wijs Method
X	...	15.8	...	2 (?)	...
X	...	18.1	...	2.5 (?)	...
X	...	13.2	...	1.5 + (?)	...
X	...	15.2	18.1	2% + (?)	2.15
X	...	9.8	8.5	1%	1.01
X	0.5	9.9	12.6	1% + (?)	1.5
X	0.75	25.04	28.4	3% + (?)	3.4
Y	1.1	5.2	2.6	0.4	0.31
Y	1.0	2.0	1.4	0.1	0.16
Y	...	17.1	15.5	2% + (?)	1.84

(?) Doubtful maximal values owing to inaccuracy of method.

CONCLUSIONS

The question now arises as to whether the standard for the unsaturated impurity should be altered so as to meet the commercial variations found or enforced so that manufacturers are forced to provide a more uniform product. It is obvious that cyclopropane can be made with less of the unsaturated impurity than occurs frequently. It is a manufacturer's problem whether this can be achieved regularly.

The dangers arising from the inclusion of even 2.5% of propylene do not seem to be great. It is true that propylene was condemned on account of the occurrence of cardiac irregularities, but at that time it was not known that these occurred even with ether, and the purest cyclopropane obtainable will also produce irregularities, as experiments in animals have shown. Cardiac deaths under cyclopropane, properly administered, do not seem to have been recorded. There seems no good reason for making the standard so rigid.

Allene is a less desirable impurity than propylene because of the lung damage it produces in anesthetic concentrations. Ferguson's experiments suggest that this would probably not occur in low concentrations such as 2%. It also produces cardiac irregularities in anesthetic concentrations.

The Supplement allows for the presence of up to 1% of gas not absorbed by sulfuric acid and this amount frequently occurs in manufacturers' samples and seems to be a fair standard. But from the above, it is evident that the estimation of unsaturated impurity, if propylene alone were present, would allow of the presence of 1% of this gas. Consequently the definition which states that "Cyclopropane contains not less than 99% of CH_2—CH_2—CH_2" should be altered to read "not less than 98%," and as commercial samples frequently contain, as the Wijs method clearly shows, more than 1%, often 2%, of unsaturated impurity, the requirement of even 98% cyclopropane would be very rigid.

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Determination of Nitroglycerin in Concentrated Triturations

*By George F. Hutchison**

Nitroglycerin for medicinal use is commonly supplied to manufacturing pharmacists either as a trituration with sugar of milk or chalk or as a solution in 95% ethanol. The concentration may vary with individual orders but is usually specified as 10% by weight. From time to time controversies have arisen between this Laboratory and the manufacturing pharmacists as to the exact percentage of nitroglycerin present in these sugar of milk triturations, in spite of the great care taken to assure a definite and uniform concentration. In view of the care used in weighing and blending the ingredients, it appears that shortages cannot ordinarily be attributed to errors on the part of the supplier.

Experience in filling orders for these ingredients over a period of years has led to the conviction that the complaints are caused chiefly by the assay methods used by the purchasers. It is conceivable that methods suitable for assay of tablets, containing $^1/_{100}$ grain each, would not be accurate for a trituration containing 10% nitroglycerin. The object of this paper is to present data supporting this statement and to propose a more practical method for assay of concentrated triturations of nitroglycerin.

The *Pharmacopœia of the United States*, 11th Revision, 1935, gives methods of assay for spirit of nitroglycerin and tablets of nitroglycerin, but offers no specific method for the concentrated mixtures. This is also true of *Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists*, 4th Edition, 1935, and *Pharmaceutical Standards, Including Tolerances and Methods of Analysis* of the American Drug Manufacturers' Association and the American Pharmaceutical Manufacturers' Association, Revised Edition, 1935.

There are few references in the literature to the determination of nitroglycerin in concentrated medicinal preparations. Several attempts have been made to use saponification with alcoholic potash as a quantitative method, but the consensus is that it is unreliable because of various side reactions. This was reviewed by Majrich (1), who presented data on the saponification of organic nitrates, indicating that they have a peroxide-type formula. However, Hay (2) found that saponification of nitroglycerin produced a uniform quantity of nitrite, determined colormetrically, even when conditions were widely varied. Heyl and Staley (3) obtained good precision in the analysis of concentrated (10%) triturations and alcoholic solutions by the Kjeldahl method, using the usual modification for nitrates. They found that the colorimetric method of Scoville (4), using phenoldisulfonic acid, was also satisfactory but considered it better adapted to small amounts, as in tablets.

Murray (5) recommended the colorimetric methods of Hay and of Scoville for assay of nitroglycerin tablets. In collaborative work by twenty laboratories (6) both methods gave good results in most cases. Sykes (7) extracted tablets with glacial acetic acid and compared colors obtained with diphenylamine, while Meek (8) used a similar extraction but depended on the color produced by phenoldisulfonic acid.

The method of the A. O. A. C. (9) depends on extraction with ether, followed by a reduction of the nitrate with Devarda's alloy and distillation of the ammonia into standard acid, using a special scrubber. It is the same as the method of the *U. S. Pharmacopœia* (10) and the preferred method of the *Pharmaceutical Standards* (11), except that they both saponify the nitroglycerin in the presence of potassium permanganate after the ether extraction.

The use of the Devarda method with the preliminary saponification was proposed by Grantham (12). It was later studied by Hanson (13, 14) who claimed that results were more nearly quantitative if the saponification was omitted. Caron and Raquet (15) also recommended the Devarda method without saponification and considered it more accurate than colorimetric methods. Popov (16) used aluminum instead of De-

* Contribution No. 23 from the Eastern Laboratory of E. I. du Pont de Nemours & Company.

varda's alloy. Anderson (17) substituted an acid distillation for the ether-extraction step, collecting the distillate in 5% NaOH and subjecting this to the usual Devarda procedure. He noted a high blank on the alkaline distillation, even though an efficient scrubber was used. This is one of the serious defects of the Devarda method for nitrates. Kenworthy (18) obtained 98.9% to 103.1% recovery of nitroglycerin by Anderson's method in the presence of other medicinal ingredients when these were added singly. Smith (19) used a similar method but substituted reduced iron for Devarda's alloy and determined ammonia in the final distillate by means of Nessler's reagent instead of by titrating the excess of standard acid.

The method which we have found most satisfactory when high concentrations of nitroglycerin are to be determined consists of extraction with pure, dry ether in a Wiley apparatus, evaporating the ether at room temperature and weighing the dried ether extract. It is similar to the method recommended by the U. S. Bureau of Mines for determination of nitroglycerin in dynamite (20). Murray (5, 6) mentioned ether extraction as a method for assay of nitroglycerin tablets and Engelhardt (21) recommended it. The details of our method are as follows:—

EXPERIMENTAL

Apparatus and Reagents.—Wiley Extraction Apparatus, complete as described in Arthur H. Thomas Company's catalog No. 5020. The Gooch crucible should be prepared in the usual way with a fairly thin asbestos mat and dried at 100° C.

Ether, Free from Alcohol and Peroxides.—It is most important that the ether be of the best quality, particularly that it be free from the impurities mentioned. Alcohol is objectionable because it is difficult to remove by evaporation after the extraction. If peroxides of ether are present some of the nitroglycerin may be decomposed toward the end of the evaporation and, in fact, when this impurity is considerable, the entire residue of nitroglycerin may decompose with evolution of brown fumes.

The formation of peroxides is caused by exposure to light, so that a freshly opened can should not show such impurities. The following test is recommended by the Institute of Makers of Explosives: Add 1 cc. of a solution of 5% cadmium iodide, 5% potassium iodide and 90% water to 10 cc. of ether in a glass-stoppered bottle. Shake several times during a period of one hour, keeping bottle in the dark. Orange color in the solution indicates peroxide.

If this test is positive, the ether may be purified by the method of Gatterman and Wieland (22) as follows: Shake in a separatory funnel with about one-tenth its volume of a solution consisting of 100 Gm. $FeSO_4.7H_2O$ and 50 cc. H_2SO_4 (96%) in a liter of water. Repeat this extraction twice, then wash three times with water, add anhydrous calcium chloride to the ether layer and distil. If peroxides are absent, the ether need only be freed from alcohol by washing three times with water in a separatory funnel, shaking with anhydrous calcium chloride and distilling.

Nitroglycerin Remover.—Dissolve 7 parts by weight of sodium sulfide (fused chips, 60% Na_2S) in 23 parts water. Add 54 parts denatured alcohol and 16 parts acetone. Mix thoroughly.

Procedure.—Weigh 4 Gm. of the concentrated trituration of nitroglycerin into the Gooch crucible, suspend it in the Wiley extractor and add 40 cc. purified ether. Pass cold water through the condenser and immerse the tube of the extractor in a water-bath heated with a steam coil. The ether should boil at such a rate that the condensate covers the sample but does not flow over the side of the crucible.

Continue the extraction for five hours, then raise the apparatus from the bath and allow the crucible to drain. Remove the glass tube from the apparatus, pour the solution into a tared 150-cc. beaker and rinse the tube with three or four 10-cc. portions of ether, adding them to the beaker. Let the beaker stand at room temperature for about sixteen hours in a place not unduly exposed to dust. If there is no odor of ether, weigh the beaker and contents and, after standing several hours longer, repeat the weighing.

To obtain results more quickly, an optional method of evaporation may be carried out by placing the beaker on the steam-bath, but care must be taken to remove the beaker when about 10 to 15 cc. remain to be evaporated. Otherwise, loss may occur because of the volatility of nitroglycerin at a temperature above that of the normal room. Moreover, heating on the steam-bath after all ether is removed may cause complete decomposition, with evolution of fumes of nitrogen peroxide.

The residue weighed represents nitroglycerin, which is calculated to per cent of the sample taken.

Disposal of Nitroglycerin.—When final weight of beaker and contents has been obtained the nitroglycerin should be disposed of at once. Add to the beaker 25 cc. of "nitrolgycerin remover," prepared as described above under "Apparatus and Reagents." Allow the beaker to stand 10 to 15 minutes and pour the solution into the sink. The sodium sulfide decomposes the nitroglycerin completely so that it no longer has explosive properties.

A comparison of this method with that recommended by the A. O. A. C. for tablets gave the results set forth in Table I.

Table I.—Results Obtained by Weighing Ether Extract and by A. O. A. C. Method

Sample No.	3	4	8	9
Weighing ether extract	10.10	9.25	11.00	9.92
	10.08	9.40	11.00	9.87
	...	...	10.99	...
A. O. A. C. method	8.81	8.28	6.76*	8.50
	8.76	8.53	10.14*	8.70
	...	...	9.30	...
	...	...	9.43	...

While it appears that the variation in the determinations marked with asterisks may have been caused by faulty manipulation, they have been included for the sake of completeness. If these are eliminated, duplicates obtained by the A. O. A. C. method are in agreement, but only show from 84.5% to 91.5% of the mean of the values obtained by weighing the ether extract. The scrubber used was an exact reproduction of one of those depicted in the reference (9) and all details were strictly observed.

Another method, which has been quoted by purchasers of medicinal nitroglycerin more than any other, is that designated as "alternate" in the *Pharmaceutical Standards* (11). In this method, the crushed tablets are heated strongly with milk sugar, aluminum sheet and 1:1 KOH solution in a copper distilling flask and boiled until the dense white fumes cease. The ammonia formed is absorbed in excess of standard acid and back titrated. We have not been able to find any data on this method in the literature. In subsequent discussion it will be referred to as the "alternate method."

Because of its wide acceptance as a method of analyzing concentrated nitroglycerin triturations, more time was spent on this than the other methods studied. The results obtained are shown in Table II, in comparison with those obtained by weighing the ether extract.

In analysis of Sample 1, five out of seven determinations by the "alternate method" agreed closely with the ether extract method; also, for Sample 2 the agreement between the two methods was satisfactory. However, this agreement was not found on later samples, all of which gave lower results by the "alternate method," ranging from 86.7% to 93.3% of the mean of the ether extracts. (Sample 7 was a trituration with chalk instead of sugar of milk.) In analysis of some of the later samples, connections of the apparatus were renewed and all details of the procedure carefully checked without improvement in the results.

In addition to the analyses shown in Table II, Sample 1 was analyzed by what may be called the preferred method of the *Pharmaceutical Standards* (11), which is essentially the same as the method of the U. S. Pharmacopœia. This gave 9.29% and 9.44%.

The "alternate method" and the ether extract method were then checked against known samples. For the "alternate method," samples of dry nitroglycerin in the approximate amount specified by the method, 0.06 Gm., were weighed by difference and dropped directly into the copper flasks containing 0.6 Gm. sugar of milk. The analysis was then conducted as usual.

For the ether extract method, 3 Gm. dry nitroglycerin, nitrogen content 18.39% by nitrometer, were weighed into a beaker containing 27 Gm. sugar of milk. The mixture was stirred in the beaker with a hard rubber spatula for some time after it appeared to be thoroughly uniform, or about ten minutes in all. It was transferred to a bottle and stored for one week with occasional shaking. Duplicate samples of 4 Gm. each were then weighed out and analyzed by the method detailed above.

Table II.—Results Obtained by Weighing Ether Extract and by the "Alternate Method"

Sample No.	1	2	3	4	5	6	7
Weighing ether extract	10.22	10.04	10.10	9.25	9.23	10.07	9.92
	10.17	10.10	10.08	9.40	9.25	10.08	10.07
"Alternate method"	9.30	10.16	9.00	8.58	8.57	8.73	9.18
	9.16	10.14	8.97	8.70	8.59	8.91	8.92
	10.05	...	...	..	..	...	8.90
	10.03	...	...	..	..	...	8.88
	10.13	...	...	..	..	...	8.91
	10.06	...	...	..	..	...	...
	10.03	...	...	..	..	...	...

The results on known amounts of nitroglycerin are shown in Table III.

Table III.—Results with Known Amounts of Nitroglycerin

	Grams Nitroglycerin Added	Found	% Found
Ether extract	0.4000	0.3935	98.4
	0.4000	0.3941	98.5
"Alternate"	0.0670	0.0614	91.6
	0.0863	0.0783	90.7
	0.0560	0.0509	90.9
	0.0563	0.0537	95.4

It is believed that ether extraction actually gives results more nearly quantitative than shown by the two analyses given in the table. A slight loss may have occurred in mixing this small quantity because of nitroglycerin adhering to the beaker and spatula. The results are sufficient to show that the method is superior to the others investigated here. In addition to the data appearing in the above tables, many other analyses have been made over a period of years. This experience with the ether extraction

method has likewise indicated that it is precise and accurate.

The deficiency of nitroglycerin found in known samples by the "alternate method" is in line with most of the analyses given in Table II. Incidentally, it is about the same as reported by purchasers who complained of shortage and based their complaint on assay by the "alternate method." The data indicate that the method cannot be sufficiently controlled to produce reliable results.

CONCLUSIONS

This investigation has been limited to those methods of assay of nitroglycerin tablets which have official status in the United States and which have been applied to the assay of concentrated triturations. It has been shown that, with a few exceptions, the official methods give low results on the concentrated triturations in comparison with the ether extract. On known samples the ether extract gave results which approached the known value more closely than results obtained by the "alternate method."

In view of these comparisons, it would seem desirable for manufacturers of nitroglycerin tablets to assay their purchases of concentrated triturations by the ether-extraction method. It appears that less error will be introduced in compounding tablets when this is done than when the concentrated product is analyzed by one of the official methods recommended for the assay of tablets.

Since many substances are soluble in ether, objection may be raised to a mere weighing of ether-soluble material as an assay for nitroglycerin. Yet the "alternate method," which has been widely used, is open to a similar criticism, because an equivalent amount of any nitrate, saltpeter, for example, would be determined as nitroglycerin. If there is doubt as to the composition of the ether extract, qualitative tests for nitrate can be made. If further confirmation is desired, the nitrogen content can be determined by the nitrometer or by Becker's method (23). Commercial nitroglycerin ordinarily contains 18.30% to 18.45% nitrogen (theoretical 18.51%).

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Permanganate and Cerimetric Methods in Pharmaceutical Assays

A Study of the Relative Merits

By Thomas A. Walb and Arthur E. James†*

INTRODUCTION

Potassium permanganate has been the most generally accepted oxidant in volumetric analysis for the past fifty years. This supremacy has been seriously challenged during the past decade by the introduction of ceric salts.

As early as 1861, Lange (1) suggested the use of acidified solutions of ceric salts as volumetric oxidizing agents. Further work on this subject was neglected until the systematic studies of Atanasiu (2, 3) in 1928. This work did not attract wide attention as it was confined to electrochemistry. Since 1928, additional developments in the use of cerimetric methods have been devised by Willard and Young (4, 5), Furman (6–10) and others. As a result of these investigations and the introduction of a satisfactory indicator by Walden (11), which eliminated the necessity of electrometric titrations, the practicability of ceric solutions as oxidants has been established.

Since much of Willard's and of Furman's work dealt with a comparison of the results obtained with the use of ceric solutions and those obtained with permanganate and dichromate solutions, and in view of the growing popularity of cerimetric methods, it appeared desirable to the authors of this paper to compare them with methods now in use in the United States Pharmacopœia.

Quadrivalent cerium is a powerful oxidizing agent in acid solutions. The normal oxidation potential (12), referred to hydrogen, is 1.46 volts (Ce^{IV} to Ce^{III}), while that of potassium permanganate (13) is 1.52 volts (Mn^{VII} to Mn^{II}) and that of potassium dichromate (13) is 1.30 volts (Cr^{VI} to Cr^{III}), each at a concentration of 1*N* sulfuric acid.

Advantages of Cerimetric Methods.—The respective merits and limitations of ceric sulfate, potassium permanganate and potassium dichromate are as follows:

1. The solutions of this salt are exceedingly stable (14).

2. Ceric solutions can be used in the presence of a high concentration of hydrochloric, sulfuric or perchloric acids. Its use in strong hydrochloric acid is a distinct advantage over potassium permanganate.

3. Ceric solutions can be standardized against the same primary standards as potassium permanganate, namely: electrolytic iron (15), sodium oxalate (16), arsenous oxide (17).

Recent announcement (18) proposes the use of a pure ceric salt, hexanitro ammonium cerate, $(NH_4)_2Ce(NO_3)_6$, which makes possible the preparation of a standard solution by direct weighing. The authors of this paper have not as yet substantiated this claim.

4. There is only one change in valence, Ce^{IV} to Ce^{III}, and therefore no intermediary products are possible.

5. The high oxidation potential has already been cited.

6. The reduced form, cerous ion, is colorless and hence offers no interference with indicators.

Disadvantages of Cerimetric Methods.—1. Until recently cerate methods were limited to electrometric titrations. While this disadvantage has been eliminated by the development of a satisfactory indicator (11), the necessity of using a catalyst is still essential in most titrations.

2. The change of color of Ce^{IV} to Ce^{III} is from yellow to colorless but the end-point is not easily discernible necessitating, in most cases, the use of an indicator.

3. Cerate methods are less economical than those involving the use of potassium permanganate or potassium dichromate.

At current prices, 1939, it costs \$0.27 to prepare 1 liter of 0.1*N* $Ce(SO_4)_2$, \$0.37 for $Ce(NH_4)_2(SO_4)_2.2H_2O$, \$0.005 for $KMnO_4$ and \$0.008 for $K_2Cr_2O_7$.

Advantages of Potassium Permanganate.—1. Its reactions generally occur with marked rapidity.

2. It serves as its own indicator.

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3. It can be used in acid, alkaline and neutral solutions.

4. It is very inexpensive.

5. It has a high oxidation potential.

Disadvantages of Potassium Permanganate.—1. It cannot be obtained pure enough to warrant its use as a primary standard.

2. The solutions of this salt must be allowed to stand for a considerable time and must be filtered before standardization is possible.

3. The oxidation reduction reactions involving this salt in acid media are limited to sulfuric and perchloric acids. The presence of hydrochloric acid is not desirable and cannot be used in the titration of ferrous iron.

4. The change in valence of potassium permanganate is Mn^{VII} to Mn^{II} with the possibility of forming compounds of variable valence, *i. e.*, $Mn,^{VI}$ Mn^{IV}, Mn^{III}, Mn^{II}.

5. Unless special precautions are taken potassium permanganate solutions are unstable and must be standardized at frequent intervals (19, 20, 21).

Advantages of Potassium Dichromate.—1. Solutions of this salt are stable over a long period of time.

2. The salt can be obtained pure enough to be used as its own primary standard.

Disadvantages of Potassium Dichromate.—1. The use of this salt as an oxidant is practically limited to the determination of iron.

2. The titrations with this salt require the use of either an external or an internal indicator. Satisfactory results are obtained by the use of an external indicator although internal indicators are receiving favorable attention (22, 23).

EXPERIMENTAL

Electrolytic iron wire (Mallinckrodt 99.86 per cent iron) was used to standardize the oxidizing solutions of ceric ammonium sulfate, potassium permanganate and potassium dichromate. Samples weighing approximately 0.2 Gm. were dissolved in enough concentrated sulfuric acid (96 per cent) to make the final volume of 200 cc. $2N$ in acid. These solutions were reduced by the use of the Jones reductor and titrated against the respective oxidizing solutions, which were approximately $0.1N$.

The following chemicals, which are typical of The United States Pharmacopœia XI assays, were assayed by each of the three oxidizing solutions where possible: Ferrous Ammonium Sulfate, Sodium Perborate U. S. P., Solution of Hydrogen Peroxide U. S. P., Calcium Bromide U. S. P., Calcium Chloride U. S. P. and Calcium Gluconate U. S. P. Since ferrous ammonium sulfate is more stable than ferrous sulfate U. S. P., this salt was included in the group of substances assayed. Because potassium dichromate could not be used in the assay of calcium bromide and of calcium chloride, the Volhard method was used for the determination of these salts.

The method used in the analysis of the salts was that of The United States Pharmacopœia XI,[1] substituting the several oxidizing solutions as required for potassium permanganate, which is the oxidizing solution in the official methods of assay.

In the use of ceric ammonium sulfate, osmium tetroxide, in the form of a $0.01M$ solution in $0.1N$ sulfuric acid, was the catalyst used (0.15 cc.), excepting the determinations involving the oxalate ion in which iodine monochloride (5 cc.) served as the catalyst.

The iodine monochloride used was prepared as follows: 0.279 Gm. of potassium iodide and 0.178 Gm. of potassium iodate were dissolved in 250 cc. of distilled water and 250 cc. of concentrated hydrochloric acid were added immediately (4).

The indicator used in all the determinations involving the ceric salt was the ortho-phenanthroline ferrous sulfate complex (11). This was prepared by dissolving the calculated quantity of ortho-phenanthroline, $C_{12}H_8N_2.H_2O$, in $0.025M$ ferrous sulfate and $0.5M$ sulfuric acid so that the final solution is $0.25M$ in respect to the indicator.

The indicator color change is:

$$Fe(C_{12}H_8N_2)_3^{++} \longrightarrow Fe(C_{12}H_8N_2)_3^{+++} + e$$

Intense red ⟵ Pale blue

The oxidation potential of the indicator is 1.14 volts producing a sharp end-point. The reaction is reversible as shown above.

The ceric ammonium sulfate determinations were carried out at room temperatures except in the case of the oxalate ion which required a temperature of about 50° C.

DISCUSSION OF RESULTS

In the determination of ferrous iron it appears that there is very little choice between the three oxidants used, as far as precision is concerned.[2]

The experimental data indicate that ceric ammonium sulfate is as precise as potassium

[1] In the analysis of calcium bromide the U. S. P. XI First Supplement method was used.

[2] In the Second Supplement to the U. S. P. XI, issued in September 1939, Pills of Ferrous Carbonate and Mass of Ferrous Carbonate are assayed by the use of ceric sulfate.

Experimental Data

Substance	Theory	Cerate	$KMnO_4$	$K_2Cr_2O_7$	Volhard
Ferrous ammonium sulfate	14.24% Fe	14.31	14.18	14.23	.....
		14.28	14.15	14.16	.....
		14.25	14.27	14.18	.....
		14.22	14.21	14.25	.....
		14.19	14.19	14.24	.....
		14.20	14.17	14.18	.....
		14.24	14.14	.....	.....
		14.34	14.17	.....	.....
	Av. % Fe	14.25	14.19	14.21	
Ferrous ammonium sulfate[a]		5.12	5.05	5.04	.....
		5.12	5.00	5.09	.....
	Av. % Fe	5.12	5.03	5.07	.....
Sodium perborate, U. S. P.	Not less than 9% free O_2, U. S. P.	9.63	9.66	.....	.....
		9.64	9.64	.....	.....
		9.66	9.64	.....	.....
		9.65	9.63	.....	.....
		9.66	9.65	.....	.....
	Av. % O_2	9.65	9.65	.....	.....
Solution hydrogen peroxide, U. S. P.	2.5 to 3.5% H_2O_2, U. S. P.	2.93	2.95	.....	.....
		2.93	2.95	.....	.....
		2.95	2.97	.....	.....
		2.96	2.96	.....	.....
		2.94	2.96	.....	.....
		2.94	2.95	.....	.....
	Av. % H_2O_2	2.94	2.96	.....	.....
Calcium bromide, U. S. P.	84.73% $CaBr_2$ (84–89% U. S. P.)	88.33	89.44	.....	88.68
		88.62	88.96	.....	88.61
		88.77	89.24	.....	88.65
	Av. % $CaBr_2$	88.57	89.21	.....	88.65
				Modified Volhard[b]	
Calcium chloride U. S. P.	75.49% $CaCl_2$ (75–85% U. S. P.)	74.15	74.45	74.19	74.04
		74.30	74.17	74.23	74.02
		74.30	74.29	74.07	74.04
		74.21	74.24	.....	.....
		74.15	74.35	.....	.....
	Av. % $CaCl_2$	74.22	74.30	74.16	74.03
Calcium gluconate, U. S. P.	12.51% CaO (12.4–12.8% CaO U. S. P.)	12.23	12.58	.....	.....
		12.22	12.46	.....	.....
		12.17	12.50	.....	.....
	Av. % CaO	12.21	12.51	.....	.....

[a] A specially prepared sample used for student analysis.

[b] The modified Volhard method using nitrobenzene eliminates the necessity of filtering. Caldwell and Moyer, *Ind. Eng. Chem., Anal. Ed.*, 7 (1935), 38.

permanganate in the determination of oxygen in solutions of hydrogen peroxide and in sodium perborate.

Although the literature[3] indicates that the toxic properties of osmium tetroxide have been recognized for a long time, none of the references dealing with cerate methods, in which this substance was used as a catalyst, mentioned precautions for handling it. A vial of osmium tetroxide was accidentally broken and the contents falling on the operator's hands caused a black deposit of metallic osmium. The fumes given off were highly irritating to the eyes and nasal passages.

The data relative to the assay of calcium bromide indicate that the method of assay as outlined in the First Supplement of the United States Pharmacopœia XI does not give as consistent results as do the cerate and the Volhard methods. Discrepancies in weighing, caused by the deliquescent nature of this salt, were eliminated by the use of aliquot samples.

[3] "Textbook of Inorganic Chemistry," J. N. Friend, Vol. IX, Part I, page 221, Griffin and Company.

Each of the four methods used to assay calcium chloride gave results which were in satisfactory agreement. The low results obtained for the analysis of this salt may be attributed to the fact that the sample was taken from a stock bottle which had been in the laboratory for several years.

The standards specified by The United States Pharmacopœia XI for calcium bromide dihydrate, 84–94% $CaBr_2$ (by theory 84.73%), and for calcium chloride dihydrate, 75–85% $CaCl_2$ (by theory 75.49%), would indicate that these salts, as obtained on the market, contain less water of hydration than their formulas show. If the dihydrates are obtained as such, and in view of the fact that they are strongly deliquescent, the query is raised as to why their present standards should not be adjusted to agree with this fact.

In the assay of calcium gluconate, it appears that potassium permanganate gives results which are in somewhat better agreement with the theoretical composition of the salt, than does the use of ceric ammonium sulfate. Since calcium gluconate monohydrate is a stable salt, it appears that the present Pharmacopœial standard, 12.4–12.8% CaO (by theory 12.51%), might properly be adjusted to the mean of limits corresponding more closely to the theoretical composition.

SUMMARY

It has been shown in this study that ceric ammonium sulfate can be substituted for potassium permanganate as an oxidant in pharmaceutical assays; however, the cost of the cerate method is such that its general substitution for potassium permanganate does not appear to be warranted at the present time.

From the viewpoint of simplicity, speed and precision the Volhard method appears to be preferable to the methods now used in the United States Pharmacopœia for the assay of calcium chloride and of calcium bromide.

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Adaptation of Assay Methods for Some N. F. Pastes*

By Wm. B. Baker and D. I. Kutzly

There are six pastes in the Sixth Edition of the *National Formulary* for which there are no assay requirements. Since these pastes are contained in an official book, it is important that standards be adopted for them, fixing definitely the content limits of their active ingredients.

In some cases the assay methods used for certain drugs and chemicals can be adapted for use in the assay of official preparations containing these ingredients. This appears to be especially feasible in the case of four of the six pastes referred to.

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I. PASTE OF BISMUTH

The assay method for Bismuth Subnitrate, U. S. P. XI, may be successfully adapted for use in the assay of Paste of Bismuth, N. F. VI, as shown by the satisfactory results obtained in the experiments which follow. The bismuth subnitrate, which is the only therapeutically active ingredient in the paste, was determined as bismuth oxide, after proper separation of the former from the rest of the paste. Substantially the same procedure may be followed for the determination of bismuth subnitrate as is carried out in the assay of Compound Ointment of Resorcinol, N. F.[1]

EXPERIMENTAL

A sample of Paste of Bismuth was prepared in accordance with the directions given in the N. F. VI. The bismuth subnitrate used in the paste was examined for purity prior to the preparation of the sample.

Assay of Bismuth Subnitrate.—The method of the U. S. P. XI was followed in the assay of Bismuth Subnitrate.

Table I.—Results of Assay of Bismuth Subnitrate by the U. S. P. XI Method

Assay	No. 1	No. 2	No. 3	No. 4
Wt. of sample in Gm.	1.00	1.04	1.00	1.01
Wt. of residue in Gm.	0.79	0.83	0.80	0.81
Per cent of Bi_2O_3	79.51	80.23	80.10	80.00

Average per cent of Bi_2O_3 = 79.96.

Assay of Paste of Bismuth.—Accurately weigh about 2 Gm. of Paste of Bismuth in a tared crucible, heat it slowly until melted and carefully ignite to constant weight. The residue of Bi_2O_3 is not less than 79 per cent of the amount of bismuth subnitrate in the paste taken for the assay.

Table II.—Results of Assay of Paste of Bismuth

Assay	No. 1	No. 2	No. 3	No. 4
Wt. of sample in Gm.	1.98	2.01	2.01	2.01
Wt. of residue in Gm.	0.46	0.47	0.47	0.47
Per cent of Bi_2O_3	23.67	23.72	23.60	23.71

Average per cent of Bi_2O_3 found in sample = 23.67
Calculated per cent of Bi_2O_3 in sample (30 × 79.96) = 23.98
Difference = 00.31
Percentage error (00.313 ÷ 23.988) × 100 = 1.30.

II. PASTE OF ZINC OXIDE

The zinc oxide contained in Paste of Zinc Oxide was determined by simple gravimetric means following its isolation from the other constituents of the paste.

[1] Wm. B. Baker and D. I. Kutzly, "Adaptation of Assay Methods for Some N. F. Ointments, III. Compound Ointment of Resorcinol," *Pharm. Arch.*, 10 (1939), 65.

EXPERIMENTAL

A sample of Paste of Zinc Oxide was prepared in accordance with the directions given in the N. F. VI. The zinc oxide used in the paste was examined for purity prior to the preparation of the sample.

Assay of Zinc Oxide.—The method of the U. S. P. XI was followed in the assay of zinc oxide.

Table III.—Results of Assay of Zinc Oxide by the U. S. P. XI Method

Assay	No. 1	No. 2	No. 3	No. 4
Wt. of sample in Gm.	1.50	1.50	1.50	1.53
Cc. of $N/1$ H_2SO_4 added	52.65	52.65	52.65	52.65
Cc. of $N/1$ NaOH required	15.79	15.90	16.02	15.12
Cc. of $N/1$ H_2SO_4 consumed	36.86	36.75	36.63	37.53
Per cent of ZnO	99.91	99.48	99.36	99.81

Average per cent of ZnO = 99.64.

Assay of Paste for Zinc Oxide.—Place about 2 Gm. of Paste of Zinc Oxide, accurately weighed, in a crucible of about 30-cc. capacity. Heat the crucible and contents *gently* over a Bunsen flame until the paste is liquefied. Gradually increase the temperature and ignite to eliminate the base of the paste. Dry the residue to constant weight, and weigh the zinc oxide.

Table IV.—Results of Assay of Paste of Zinc Oxide

Assay	No. 1	No. 2	No. 3	No. 4
Wt. of sample in Gm.	2.00	2.00	2.00	2.00
Wt. of residue in Gm.	0.49	0.49	0.49	0.49
Per cent of ZnO	24.87	24.81	24.81	24.85

Average per cent of ZnO found in sample = 24.84
Calculated per cent of ZnO in sample (25 × 99.64) = 24.91
Difference = 00.07
Percentage error (00.07 ÷ 24.91) × 100 = 0.28.

III. PASTE OF ZINC OXIDE WITH SALICYLIC ACID

A sample of Paste of Zinc Oxide with Salicylic Acid was prepared in accordance with the directions given in the N. F. VI. The zinc oxide and the salicylic acid used in the paste were examined for purity prior to the preparation of the sample.

EXPERIMENTAL

Assay of Zinc Oxide.—The zinc oxide used was the same material as was used in the preparation of the sample of Paste of Zinc Oxide, above. The results of the assay are shown in Table III.

Assay of Salicylic Acid.—The method of the U. S. P. XI was followed in the assay of salicylic acid.

Table V.—Results of Assay of Salicylic Acid by the U. S. P. XI Method

Assay	No. 1	No. 2	No. 3	No. 4
Wt. of sample in Gm.	0.55	0.53	0.54	0.51
Cc. of $N/10$ $Ba(OH)_2$ used	40.25	38.58	39.53	36.78
Per cent of $C_7H_6O_3$	99.51	99.05	99.58	98.90

Average per cent of $C_7H_6O_3$ = 99.26.

Assay of Paste for Zinc Oxide.—The zinc oxide was determined by simple gravimetric means following its isolation from the other constituents of the paste.

Place about 5 Gm. of Paste of Zinc Oxide with Salicylic Acid, accurately weighed, in a 250-cc.

glass-stoppered Erlenmeyer flask. Add 50 cc. of ether to the flask and shake vigorously to dissolve the salicylic acid and the white petrolatum. Filter and continue the extraction with 40, 20, 20-cc. portions of ether. Combine the filtrates and set aside for the determination of salicylic acid as directed below under "Assay of Paste for Salicylic Acid." Place filter paper and contents into a tared crucible and wash the flask thoroughly with several small portions of dilute ammonia water, combining the washings with the contents of the crucible. Heat the crucible and contents carefully on a water-bath to evaporate to dryness, continue the heating with a Bunsen flame, ignite, dry to constant weight and weigh the zinc oxide.

Table VI.—Results of Assay of Paste of Zinc Oxide with Salicylic Acid for Zinc Oxide Content

Assay	No. 1	No. 2	No. 3	No. 4
Wt. of sample in Gm.	4.85	5.03	5.00	5.02
Wt. of residue in Gm.	1.18	1.23	1.22	1.23
Per cent of ZnO	24.42	24.50	24.50	24.54
Average per cent of ZnO found in sample				= 24.49
Calculated per cent of ZnO in sample (25 × 99.64)				= 24.91
Difference				= 00.42

Percentage error (00.42 ÷ 24.91) × 100 = 1.68.

Assay of Paste for Salicylic Acid.—Evaporate the filtrate, collected in the preceding assay, by means of a current of dry air. Dissolve the residue in 50 cc. of neutral diluted alcohol. Titrate the alcoholic solution with *N*/10 sodium hydroxide, using phenolphthalein T.S. as the indicator. If the white petrolatum in the solution congeals before the titration is complete, warm on a water-bath, and continue the titration until a permanent pink color is obtained. Each cc. of tenth-normal sodium hydroxide is equivalent to 0.01381 Gm. of $C_7H_6O_3$.

Table VII.—Results of Assay of Paste of Zinc Oxide with Salicylic Acid for Salicylic Acid Content

Assay	No. 1	No. 2	No. 3	No. 4
Wt. of sample in Gm.	4.85	5.03	5.00	5.02
Cc. of *N*/10 NaOH used	6.90	7.20	7.00	6.95
Per cent of $C_7H_6O_3$	1.97	1.98	1.94	1.92
Average per cent of $C_7H_6O_3$ found in sample				= 1.95
Calculated per cent of $C_7H_6O_3$ in sample (2 × 99.26)				= 1.98
Difference				= 00.028

Percentage error (00.028 ÷ 1.985) × 100 = 1.41.

IV. HARD PASTE OF ZINC OXIDE

The zinc oxide in Hard Paste of Zinc Oxide was determined titrimetrically following its isolation from the benzoinated lard in the paste.

EXPERIMENTAL

A sample of Hard Paste of Zinc Oxide was prepared in accordance with the directions given in the N. F. VI. The zinc oxide used in the paste was examined for purity prior to the preparation of the sample.

Assay of Zinc Oxide.—The zinc oxide used was the same material as was used in the preparation of the sample of Paste of Zinc Oxide, above. The results of the assay are shown in Table III.

Assay of Paste for Zinc Oxide.—Place about 2 Gm. of Hard Paste of Zinc Oxide, accurately weighed, in a crucible of about 30-cc. capacity. Heat the crucible and contents *gently* over a Bunsen flame until the paste is liquefied. Gradually increase the temperature and ignite to free of carbonaceous material. Digest the residue remaining in the crucible after ignition with 50 cc. of normal sulfuric acid until solution is complete. Then titrate the excess of sulfuric acid with normal sodium hydroxide, using methyl orange T.S. as the indicator. Each cc. of normal sulfuric acid corresponds to 0.04069 Gm. of ZnO.

Table VIII.—Results of Assay of Hard Paste of Zinc Oxide for Zinc Oxide Content

Assay	No. 1	No. 2	No. 3	No. 4
Wt. of sample in Gm.	2.08	1.98	2.04	2.03
Cc. of *N*/1 H_2SO_4 added	53.07	52.76	52.18	54.86
Cc. of *N*/1 NaOH required	40.31	40.52	40.62	42.40
Cc. of *N*/1 H_2SO_4 consumed	12.76	12.24	12.56	12.46
Gm. of ZnO per 100 Gm. of paste	24.90	25.00	24.98	24.86
Average per cent of ZnO found in sample				= 24.94
Calculated per cent of ZnO in sample (25 × 99.64)				= 24.91
Difference				= 00.03

Percentage error (00.03 ÷ 24.91) × 100 = 0.12.

DISCUSSION

For the most part, the determinations of the active ingredients in the pastes studied were carried out without difficulty after proper isolation from their respective bases. The actual separation of the active ingredients from their bases presented little difficulty.

In obtaining the residue of bismuth oxide, during the assay of Paste of Bismuth, the ignition should be carried out carefully, in order to prevent fusion and subsequent formation of metallic bismuth.

The methods described above give accurate and consistent results. Therefore, it is recommended that they be adopted for admission to the *National Formulary*, and that the following standards be prescribed:

Paste of Bismuth.—Paste of Bismuth contains not less than 28 per cent and not more than 32 per cent of bismuth subnitrate, equivalent to not less than 22.12 per cent and not more than 25.28 per cent of Bi_2O_3.

Paste of Zinc Oxide.—Paste of Zinc Oxide contains not less than 23 per cent and not more than 27 per cent of ZnO.

Paste of Zinc Oxide with Salicylic Acid.—Paste of Zinc Oxide with Salicylic Acid contains not less than 23 per cent and not

more than 26 per cent of ZnO, and not less than 1.8 per cent and not more than 2.2 per cent of $C_7H_6O_3$.

Hard Paste of Zinc Oxide.—Hard Paste of Zinc Oxide contains not less than 23 per cent and not more than 27 per cent of ZnO.

Assays for Iodine and Iodide in Iodine Solutions*

By Berl S. Alstodt

The iodine solutions considered in this study are the U. S. P. XI preparations: Tinctura Iodi, Tinctura Iodi Mitis and Liquor Iodi Compositus. The proposed assays are applicable also to other iodine-iodide solutions, such as Tinctura Iodi Fortior, N. F. VI.

The United States Pharmacopœia XI directs that the iodine of iodine solutions be determined by titrating a 5-cc. portion with *N*/10 sodium thiosulfate. To determine the iodide, the Pharmacopœia directs that another 5-cc. portion be evaporated on a water-bath to dryness, that it be repeatedly moistened until a white residue remains, which is weighed and calculated as potassium iodide for Tinctura Iodi and Liquor Iodi Compositus, or as sodium iodide for Tinctura Iodi Mitis.

The British Pharmacopœia, 1932, directs that the iodine be determined by titrating a 10-cc. portion of the iodine solution with *N*/10 sodium thiosulfate; and the potassium iodide by treating another 10-cc. portion according to the method of Andrews (1).

The thiosulfate titration for determining iodine is generally regarded as satisfactory and requires no further discussion. The iodide assay according to U. S. P. XI, although simple, is definitely unsatisfactory for several reasons. First, the method does not determine the iodide only, but rather total solids on evaporation. This was pointed out by Schaefer (2). Second, the method is time-consuming, requiring as many as twenty to thirty moistenings, over a period of three to five hours. Schaefer, in his article, states that very often it is difficult to obtain a white residue, due to impurities present. Third, the method invariably leads to high results. From Table II (see Experimental Part) it can be seen that the potassium iodide by the U. S. P. XI method exceeds the theoretical amount by four to six parts per thousand; and exceeds the iodate method by about four parts per thousand. In the case of sodium iodide, by the U. S. P. method, the theoretical amount is exceeded by about one hundred and thirty parts per thousand; and with the iodate method, it is exceeded by about one hundred and twenty parts per thousand. Fourth, the use of a 5-cc. portion in the assay of Tinctura Iodi Mitis results in decreased accuracy, since only about 7 cc. of the standard solution are used in the titration, and only about 110 mg. of the difficultly weighable sodium iodide are obtained.

The high results in the iodide content when determined by U. S. P. XI method led to the belief that not all of the water which is added in order to volatilize the iodine to obtain a white residue is removed by heating on the water-bath. The white residue obtained by the pharmacopœial method was therefore dried for two hours in an electric oven at 110° C. Table II (see Experimental Part) shows that the amounts of potassium iodide and sodium iodide were considerably decreased. This, obviously, must be due to water not removed by heating on the water-bath only. The potassium iodide content by the official method is about six parts per thousand higher than the potassium iodide content obtained after drying at 110° C. for two hours.

As previously indicated, the sodium iodide assay in Tinctura Iodi Mitis is even less satisfactory. The deliquescent nature of sodium iodide renders the attainment of close checks almost impossible. This is indicated by the relative average deviation of fifty-one parts per thousand, for the series of determinations of sodium iodide made by the U. S. P. XI method. Table II (see Experimental Part) shows the diversity of results which may be expected when the sodium iodide is determined by weighing the residue from a 5-cc. sample of the mild tincture.

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The British Pharmacopœia, 1932, directs that potassium iodide be determined by titrating with $M/20$ potassium iodate and thus obviates the difficulties encountered with the U. S. P. XI method. However, the B. P. method necessitates the use of separate portions for the iodine and iodide determinations.

A single sample is much to be preferred, as has been shown by Schaefer (2), and by Matthes and Brause (3). In this study a single sample is used for analysis. The iodine is determined by means of $N/10$ sodium arsenite. When the iodine is decolorized, the solution is rendered sufficiently acid, so that it is at least $3N$ at the end of the titration. After being rendered acid, the solution is titrated with $M/20$ potassium iodate until the iodine color is removed from the chloroform, a few cc. of which are added just before the end-point is reached.

In the titration with sodium arsenite no starch test solution is used. The end-point is readily discernible without this indicator. If, however, the starch indicator is preferred to indicate the complete removal of iodine, not more than 1 cc. should be used. It has been found that when too much of the starch indicator is added, it interferes somewhat with detecting the complete removal of the iodine in the chloroform layer. This is due to a turbidity produced in the chloroform. Kolthoff (4) points out that the sensitivity of the detection of free iodine in chloroform exceeds that of the conventional starch indicator and renders a correction factor for the indicator unnecessary.

In the sodium arsenite titration, it is important that enough sodium bicarbonate be added to present the reversibility of the reaction:

$$H_2AsO_3^- + I_2 + H_2O \longrightarrow H_2AsO_4^- + 2H^+ + 2I^-$$

The addition of an excessive amount of alkali must be avoided, for too great alkalinity will result in converting some iodine to iodide and hypoiodite or iodate.

$$OH^- + I_2 \longrightarrow HIO + I^-$$
$$12OH^- + 9I_2 \longrightarrow 2HIO_3^- + 16I^- + 6H_2O$$

Washburn (5) has calculated that optimum results are obtained at a p_H range between 4 and 9.

Reinthaler (6) and Kolthoff and Sandell (7) point out that an alkaline solution of arsenous acid will on standing be gradually oxidized by exposure to the atmosphere. According to Reinthaler (6), the oxidation is quite rapid at elevated temperatures, but at ordinary temperatures no perceptible change takes place in the titer in a period of four months. The sodium arsenite solution was standardized from time to time during this study over a period of three months and no discernible change in the titer was observed. It is recommended, however, that the sodium arsenite solution be standardized from time to time against a standard solution of potassium iodate, which according to Jamieson (8) will keep its strength indefinitely. Jamieson observed no perceptible change in a solution of potassium iodate, properly kept, in a period of ten years.

In titrating with potassium iodate, the standard solution should be added with vigorous shaking until the last drop decolorizes the chloroform layer that collects in the neck of the inverted flask. Near the end of the titration, the flask must be closed and thoroughly shaken after the addition of each drop of the standard solution (9). This may appear rather tedious. However, with experience it is quite easy to determine from the color of the solution and from the intensity of the iodine color in the chloroform how much of the standard solution is necessary.

Tables I and II (see Experimental Part) indicate that the suggested procedure is sufficiently accurate and precise for pharmacopœial assays. The precision and accuracy of the suggested procedure in each instance equal or surpass the precision and accuracy of the U. S. P. XI.

EXPERIMENTAL

Iodine Solutions.—A liter of each of Tinctura Iodi, Tinctura Iodi Mitis and Liquor Iodi Compositus was prepared using reagent quality iodine, reagent quality potassium iodide and U. S. P. sodium iodide. The reagents were weighed to the nearest 0.1 mg. and the total volume was exactly 1 liter. In each instance, the chemicals used were assayed according to the U. S. P. XI and the following values were obtained:

Iodine	99.87 ± 0.09 per cent.
Potassium iodide	99.93 ± 0.04 per cent.
Sodium iodide	97.74 ± 0.04 per cent.

Table I.—Results of Assays for Iodine

	Iodine Content in Gm. per 100 Cc.		
	Tr. Iodi	Tr. Iodi Mitis	Liq. Iodi Compositus
Theoretical	6.991 ± 0.006	1.997 ± 0.002	4.994 ± 0.005
U. S. P. XI Method	6.967 ± 0.007	1.983 ± 0.005	4.989 ± 0.005
Arsenite method	6.974 ± 0.007	1.993 ± 0.004	5.001 ± 0.003

Table II.—Results of Assays for Iodide

	Iodide[a] Content in Gm. per 100 Cc.		
	Tr. Iodi	Tr. Iodi Mitis	Liq. Iodi Compositus
Theoretical	4.997 ± 0.002	2.248 ± 0.001	9.993 ± 0.004
U. S. P. XI method	5.019 ± 0.015	2.539 ± 0.130	10.05 ± 0.01
U. S. P. XI method[b]	4.990 ± 0.007	2.299 ± 0.009	9.994 ± 0.017
Iodate method	4.999 ± 0.007	2.269 ± 0.005	10.01 ± 0.01

a Potassium Iodide in Tr. Iodi and Liq. Iodi Comp.; Sodium Iodide in Tr. Iodi Mitis.
b U. S. P. XI method modified by drying for two hours at 110° C.

M/20 Potassium Iodate.—This solution was prepared according to U. S. P. XI.

N/10 Sodium Arsenite.—Reagent quality arsenous oxide was dried for one hour at 110° C. and assayed. The assay value was 99.98 per cent. 5.0000 Gm. of the dried arsenous oxide were dissolved in 40 cc. of 5*N* sodium hydroxide. The solution was diluted to 400 cc., rendered neutral with 1*M* hydrochloric acid and then made just slightly acid. To the solution were added 2 Gm. of sodium bicarbonate and when this was dissolved the solution was made up to 1 liter. The theoretical titer of this solution was 0.10108*N*. The *N*/10 sodium arsenite was then standardized against *M*/20 potassium iodate and was found to be 0.10115*N*. Since an accuracy of one per thousand was regarded as sufficient and since the sodium arsenite solution was to be subsequently used against potassium iodate, the titer used was 0.1012*N*.

PROCEDURE

Tinctura Iodi and Liquor Iodi Compositus.—Transfer 5 cc. of the solution accurately measured with a pipette to a 250-cc. conical flask provided with a ground-glass stopper (iodine flask). Add 20 cc. of distilled water and 0.5 Gm. of sodium bicarbonate. Titrate at once with vigorous shaking with the standard solution of sodium arsenite added dropwise, until the solution turns colorless. One cc. of *N*/10 sodium arsenite is the equivalent of 0.01269 Gm. of iodine.

Cool the colorless solution under running water, add 50 cc. of 12*M* hydrochloric acid, and again cool the solution. Titrate with *M*/20 potassium iodate, shaking vigorously until the solution becomes light brown in color. Add 5 cc. of chloroform and continue titration by dropwise addition and vigorous shaking until the last drop decolorizes the chloroform layer. Toward the end of the titration, the flask must be closed and thoroughly agitated after the addition of each drop of the standard solution.

From the cc. of *M*/20 potassium iodate subtract the cc. of *N*/10 sodium arsenite previously required; multiply by 1.660 and divide by the volume of solution taken for analysis.

Tinctura Iodi Mitis.—Proceed as directed above, using a 10-cc. portion of the sample for analysis. Since mild Tincture of Iodine contains sodium iodide and not potassium iodide, subtract from the cc. of *M*/20 potassium iodate the cc. of *N*/10 sodium arsenite, used in the iodine determination, multiply by 1.499 and divide by the volume of the mild tincture used in the analysis.

SUMMARY

1. The U. S. P. method for the determination of the iodide in the official iodine solutions is not satisfactory and especially is this so for Tinctura Iodi Mitis.

2. The white residue of potassium iodide or sodium iodide obtained by the U. S. P. method should be subsequently dried for two hours at 110° C.

3. The suggested procedure for the determination of iodine and iodide requires only a single portion; it is more rapid and more accurate than the U. S. P. XI method.

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The Melting Point of Nicotinic Acid*

By Reidar Gording† and Leo A. Flexser‡

Within comparatively recent years, various investigators have reported widely different melting points for nicotinic acid, ranging from a value of 230–232° C. by McElvain, Conant and Corson (1) to 236–237° C. by Späth and Spitzer (2), while still more recent workers have obtained values as high as 238–239° C. (personal communication to the authors). The U. S. Pharmacopœia XI (3) states that "nicotinic acid melts between 234° and 237° C.," which is a rather wide range for a substance specified to have a purity of "not less than 99.5%."

In order to clarify these discrepancies an investigation was undertaken of the melting points of samples of nicotinic acid U. S. P. supplied by different manufacturers. In addition, one of the samples was subjected to six different purification steps, as outlined below, the melting point being checked after each step.

EXPERIMENTAL

Apparatus and Method.—Because of the difficulty encountered in regulating the U. S. P. melting-point apparatus to the desired temperature rise of $^1/_2$° C. per minute, a much larger mechanically stirred liquid bath was used and it was heated electrically rather than by a free flame. The bath consisted of a 1500-cc. beaker filled with coconut oil, this oil darkening less on repeated heating than other oils such as cottonseed, peanut or corn. Five turns of $^1/_{16}$-inch wide nichrome ribbon were wound around the lower third of the beaker and kept in place with a cement made from asbestos fiber and water glass. The current through the ribbon was controlled by a rheostat. The oil was vigorously stirred by a motor-driven laboratory stirrer. With this set-up, the temperature rise of $^1/_2$° C. per minute in the neighborhood of the melting point could be maintained with ease.

The thermometer used was an Anschutz type having a range from 200° to 270° C., graduated in 0.2°, and certified by the United States Bureau of Standards to have a correction of +0.1 at 225.0° C. and 0.0 at 250.0° C. for total immersion of bulb and liquid column. After checking against an auxiliary thermometer at 225°, 230°, 235° and 239° C., and obtaining consistent readings throughout this range on both thermometers, it was assumed that the Bureau of Standards thermometer gave correct readings in the range 230–240° C. The melting points were always determined with bulb and liquid column completely immersed in the oil, thereby eliminating the necessity for making an exposed stem correction.

The capillary tubes were carefully selected to have an internal diameter of 1.0 ± 0.1 mm., and a wall thickness of about 0.1 to 0.2 mm. They were filled to a height of 3 mm. after firmly tapping down. (The samples of nicotinic acid were all in the form of fine powders.) The capillaries were fastened to the thermometer with fine copper wire and were always long enough to stick out above the surface of the oil for a distance of several centimeters, even though the thermometer itself was totally immersed as regards bulb and liquid column.

In determining the melting point the temperature was raised rapidly to about 210° C., then heating was continued at approximately 3° C. per minute until the temperature was about 232° C., and from that point on heating was continued at exactly $^1/_2$° per minute until the melting was complete. The rate of heating was always checked with a stop-watch because a faster rise tends to give high values. The total time required for the determination was usually about one-half hour. Three readings were taken of the melting. First, the point at which the substance just began to soften, shrink or collapse, with no meniscus as yet visible; second, the point at which a clear meniscus could be seen and both solid and liquid were present in approximately equal amounts; third, the point at which all the solid had just melted and the liquid was completely clear. These points will be referred to as beginning, middle and end of melting, respectively.

Nicotinic Acids.—Samples of U. S. P. quality supplied by four different manufacturers were used. These will be referred to as Nicotinic Acid *A*, *B*, *C* and *D*.

Sample *D*, considered to be an excellent commercial sample, was subjected to the following successive steps of purification:

I. Recrystallization from water (1 in 15) after treatment with 1% Norite. Dried at 80° C.

II. Recrystallization of I from absolute alcohol (1 in 30) after treatment with 2% Norite. Dried at 80° C.

III. Dissolved II in water containing slightly more than an equivalent weight of sodium hydroxide, treated the solution with 1% Norite and evaporated until crystallization occurred. The sodium nicotinate was filtered off, washed with water and alcohol. It was then redissolved in water and hydrochloric acid added until the *p*H was 3.5. The precipitated nicotinic acid was filtered, washed and dried at 100° C.

IV. Nicotinic acid III was dissolved in hot concentrated hydrochloric acid and the solution allowed

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to cool and crystallize. The nicotinic acid hydrochloride was washed with water and air dried. It was then dissolved in water and sodium hydroxide added until the *pH* was 3.5. The precipitated nicotinic acid was washed and dried at 85° C.

V. Nicotinic acid IV was recrystallized from absolute alcohol (1 in 25). Dried at 80° C.

VI. Nicotinic acid V was sublimed *in vacuo*, heating in an oil bath at about 235° C.

These purified samples will be referred to as Nicotinic Acid I, II, III, IV, V and VI, respectively.

All of the samples were used as fine powders and all were dried in a desiccator over sulfuric acid for at least 24 hours before taking the melt. It was found, however, that this drying had no effect on the melting point, nor did drying at 100° C. for two hours have any effect.

Table.—Melting Point Data

Nicotinic Acid	Melting Point, ° C.		
	Beginning	Middle	End
A	232.0	235.9	236.5
B	232.0	235.9	236.5
C	231.8	235.5	236.4
D	232.2	236.0	236.6
I	232.0	236.0	236.4
II	232.0	236.0	236.6
III	232.0	235.8	236.2
IV	232.0	235.8	236.4
V	232.0	236.1	236.6
VI	231.0	236.1	236.6

It will be seen that all the commercial and purified samples melt over the same ranges, and that further purification of *D* by various methods which should have removed impurities did not change the melting point, indicating that the commercial material is already highly pure.

Although all of the samples softened at about 232° C., this temperature should not be considered as being in the melting range since no liquid melt will form at this temperature within a reasonable time. The true melting point where solid and liquid are in equilibrium probably lies between 235.5° and 236.6° C.

The low value obtained by McElvain, Conant and Corson (1) is probably due to the presence of nitro compounds obtained in the action of nitric acid on nicotine (4). The value of 236–237° C. reported by Späth and Spitzer (2) who sublimed their material *in vacuo* is substantially in agreement with our values.

In view of the fact that higher melting points for nicotinic acid have also been reported, it was thought that our values might be low because of decomposition during the half-hour heating time. To test this possibility, the oil bath was heated to 235° C. before placing the capillary in it and the total time required to take the melt was only 5 minutes. Values of 236.2° and 236.6° C. were obtained on sample *D* for the middle and end of melting, indicating that decomposition is probably not a significant factor.

The reason for the higher values reported is most likely due to too rapid heating during melting or to the use of a too large capillary, or an air bath rather than an oil bath. Heating sample *D* in the oil bath at a rate of 2° per minute gave values of 236.4° and 237.1° C. for the middle and end of melt, about ½° too high. The use of an electrically heated air bath (5) gave values in agreement with the oil bath only when the rate of heating did not exceed ½° per minute. When the rate of heating was 2° per minute, the middle and end of melt were 236.4° and 237.8° C., the latter figure being more than a degree too high. When the size of the capillary was increased to a diameter of 1.2 mm., filled to a depth of 7 mm. (instead of 3 mm.) and it was heated in the air bath at 2° per minute, values of 237.2° and 239.2° C. were obtained for the middle and end of melting, the latter figure being 2.5° too high. Nicotinic acid seems to be a substance either of very low heat conductivity or very high specific heat, or possibly it combines both properties. In any event, a heating rate not in excess of ½° per minute is needed to obtain the correct melting point.

Grateful acknowledgment is made to E. Dowzard of this company for helpful suggestions and criticisms throughout the course of this work.

SUMMARY

1. The melting points of various commercial and purified samples of nicotinic acid have been determined.

2. The true melting point of nicotinic acid is probably between 235.5° and 236.6° C.

3. In determining the melting point of nicotinic acid, care must be taken to heat not faster than ½° per minute or high values will be obtained.

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NOTICE

Authors are urged to return corrected proof promptly to avoid unnecessary delay in the appearance of succeeding numbers of the JOURNAL.

The Detection of Carbon Monoxide in Medicinal Oxygen

*By Frederick K. Bell and John C. Krantz, Jr.**

In an earlier communication (1) the authors described a method for the detection of carbon monoxide in medicinal oxygen. This method was based on the reduction of palladium chloride by carbon monoxide and the subsequent formation of molybdenum blue and was found to be sensitive to 10 parts per million of carbon monoxide. It was also found that this test responds to hydrogen so that modification was essential since some medicinal oxygen is prepared electrolytically and may therefore contain several tenths of a per cent of hydrogen, an impurity which is quite permissible for pharmacopœial purposes.

Our attention was first directed to the problem of altering the palladium test solution so that it might, in effect, react specifically with carbon monoxide. This mode of attack seemed promising in view of the work of Daller (2). Our efforts along this line were unsuccessful probably owing to the fact that in rendering the palladium solution practically inactive toward hydrogen, its reactivity toward the carbon monoxide was greatly reduced so that for the detection of small amounts of carbon monoxide the element of time became significant. Our original test, in the absence of hydrogen, required approximately twelve hours and any considerable lengthening of this time period would render the test impractical. We have also tried, without success, a number of methods for the removal of hydrogen from the gas mixture.

METHOD

In the face of these negative results, our attention was directed to another line of attack. The method we have devised is presented in this communication and may be briefly summarized as follows. A liter sample of the oxygen to be tested, to which have been added 50 cc. of nitrogen, is treated in a closed system with an alkaline solution of sodium hydrosulfite which removes most of the oxygen. Nitrogen is added to the residual gas until the volume is 100 cc. This 100-cc. volume, consisting mainly of nitrogen, is then tested by the hemoglobin method. A sample of carbon monoxide-free oxygen treated in the same manner serves as a standard.

The pure carbon monoxide used for the carbon monoxide–oxygen mixture was prepared by the reaction between formic and sulfuric acids. A stock mixture of 10 per cent carbon monoxide in oxygen was then prepared and the required amount of this stock mixture was introduced into a twenty-liter gasometer to give the final desired carbon monoxide–oxygen mixture. We have not analyzed these final mixtures, but their entire preparation has been repeated a number of times in the course of the work and consistent results have been obtained. A sample of analyzed carbon monoxide–oxygen mixture prepared in another laboratory was also found to check closely the results obtained from our own mixtures.

A cylinder of carbon monoxide-free oxygen, which was generously supplied by The Linde Air Products Company, was employed not only in preparing the carbon monoxide–oxygen mixtures but also for the blank determinations. A cylinder of commercial nitrogen was used.

With regard to the hemoglobin test, blood samples from a number of different sources were used. These include oxalated bloods from humans, dogs, rabbits and chickens and also defibrinated beef and sheep's blood. All of these bloods seem to have the same sensitivity. Oxygenation of the blood, before use, appears to have no effect on the sensitivity. The blood should be used within 24 hours after drawing and should be kept refrigerated until ready for use. As might be expected, hydrogen has no effect on the test, and we have verified this fact in our examination of a number of oxygen mixtures, with and without carbon monoxide, containing up to 0.5 per cent of hydrogen.

The experimental procedure can be carried out with the simplest equipment and we present it in its original form. Doubtless a number of refinements will present themselves especially in adapting the method to the requirements of a plant control laboratory.

Alone-liter, short ring neck, round-bottom Pyrex balloon flask, as shown in the above figure, is equipped with a tightly fitting two-holed rubber stopper. The capacity of the flask to the stopper was found to be approximately 1050 cc. The flask is filled with water and inverted over a pneumatic trough. Fifty cubic centimeters of nitrogen are introduced into the flask and then the remaining water, 1000 cc., is completely displaced by the oxygen to be tested.

The flask is then tightly closed with a rubber stopper bearing two glass stop-cocks, one of which is of the capillary type bearing a right-angle bend for convenience. In inserting this stop-cock through the rubber stopper the end of the arm should reach just to the lower surface of the stopper.

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With both stop-cocks closed, the flask is removed from the trough and placed in its normal position. Through the vertical stop-cock, to which a funnel has been attached, is introduced a freshly prepared solution (3) of 50 Gm. of sodium hydrosulfite in 250 cc. of distilled water to which have been added 40 cc. of a solution of 500 Gm. of potassium hydroxide in 700 cc. of water. The oxygen absorption commences with the introduction of the first few drops of the solution and the entire volume of the hydrosulfite solution can be drawn into the flask, aided by occasional shaking, within a few minutes. With both stop-cocks closed, the flask is shaken vigorously for five minutes. Distilled water is then

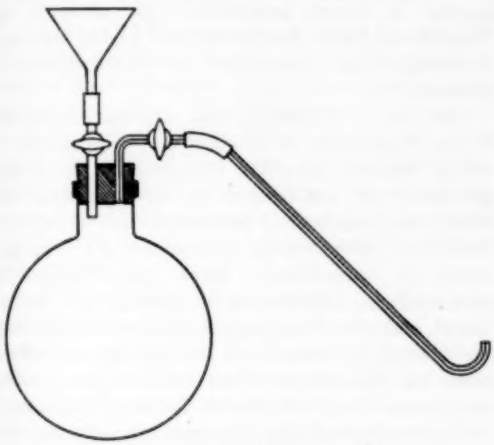

Fig. 1.—Flask and Attachments.

allowed to flow into the flask through the vertical stop-cock until atmospheric pressure is restored therein.

The residual gas volume of approximately 75 cc. is then transferred to a 100-cc. volumetric flask which has been filled with water and inverted over a pneumatic trough. To the capillary stop-cock is attached a delivery tube of capillary tubing for the transfer of the gas to the volumetric flask and the distilled water is introduced through the other stop-cock to displace the residual gas from the balloon flask. After complete transfer of the gas to the volumetric flask the balance of the water therein is completely displaced by nitrogen and the flask is then stoppered. Throughout the procedure, care should be taken to avoid the introduction of air into the gas sample.

A sample of carbon monoxide-free oxygen is carried through the same procedure and we then have two 100-cc. volumetric flasks corresponding, respectively, to the test gas and to the carbon monoxide-free oxygen.

To each of the flasks are added 2 cc. of a freshly prepared aqueous solution of 1 cc. of blood diluted to 20 cc. The flasks are immediately restoppered and, during a period of fifteen minutes, are rotated from time to time to facilitate maximum contact between the blood solution and the gas.

The blood solutions are then treated by the standard pyrotannic method in which one cc. of a fresh solution containing 1 Gm. of pyrogallic acid and 1 Gm. of tannic acid in 50 cc. of distilled water is added to each flask. After thorough mixing the flasks are allowed to stand in subdued light for 15 minutes after which time the contents of the flasks are transferred to small similar test-tubes for comparison. If the test gas contains 5 parts per million of carbon monoxide, the contents of the corresponding tube will show a pinkish tint in contrast to the brown color of that of the carbon monoxide-free sample.

SUMMARY

1. A method for the detection of carbon monoxide in medicinal oxygen has been described.

2. The method which involves a very simple procedure is considerably more rapid than the methods hitherto available and is sensitive to approximately 5 parts per million of carbon monoxide.

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A Study of the Extraction of Astringent Drugs*

By H. F. Lefevre† and C. O. Lee‡

Methods of drug extraction have been the subject of much pharmaceutical research. Recent work upon the problem has involved drugs containing alkaloids or other constituents which are rather easily assayed. The astringent drugs and their preparations are not easily standardized because their astringency is attributed to their tannin content for which there is no satisfactory assay.

This study was undertaken for the purpose of applying some of the more recently acquired knowledge of the subject of drug extraction to the preparation of astringent fluidextracts and to establish the following objectives:

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1. To determine the method, apparatus and menstruum best suited for the extraction of tannins from the astringent drugs.

2. To study the possibility of preparing full-strength astringent fluidextracts without the collection of weak percolates.

3. To show the proportion of tannin lost in the astringent galenicals over stated periods of time.

Most of the experimental work embodied in this investigation has been performed on powdered krameria root since this drug was suggested as representative of the astringent group.

EXPERIMENTAL

Assay Methods.—Inasmuch as the preparations studied depended upon the presence of tannins for their astringency, it was necessary to adopt a method of tannin assay. A modified Loewenthal-Proctor method (1) was accordingly investigated and found to be the one most suitable for this purpose and has been used exclusively in this study.

For the determination of total extractives the method used by Arnett and Lee (2) in their study of the astringent galenicals was adopted. The procedure was as follows: 10 ml. of the galenical were pipetted into a tared 2-inch porcelain evaporating dish. The dish was then placed in an electric oven and the contents dried to constant weight at 105°. The weight of the residue, multiplied by ten, gave the percentage of total extractive, weight/volume.

Menstruum Variations in the Manufacture of Fluidextract of Krameria.—Some doubt has been cast upon the value of glycerin in drug extraction. Scoville (3) expressed the opinion that although glycerin is valuable for the prevention of precipitation in fluidextracts, it has been largely overrated for that purpose. However, it appeared from his investigations that glycerin was of value in the extraction of the astringent drugs. Husa and Magid (4) found that glycerin retarded extraction in the case of belladonna root.

Table I.—Results of Tannin Assays

No.	Alcohol	Glycerin	Water	Percentage of Tannin N. F. VI Process	First 500 Ml.
1	9	1	.	18.96	16.5
2	8	2	.	19.5	16.0
3	9	.	1	19.97	17.2
4	6	.	4	18.95	15.5
5	2	.	8	17.0	14.7

Fluidextracts were prepared from a number 20 powder of krameria root, using cylindrical Pyrex glass tubes 150 cm. long and 51 mm. uniform inside diameter as the percolators. The National Formulary VI menstruum I for the preparation of Fluidextract of Krameria was varied by changes in alcohol, glycerin or water content. The results of the tannin assays made upon the fluidextracts so prepared are shown in Table I.

Comments: The substitution of water for the one part of glycerin increased the amount of tannin in the fluidextract prepared by the National Formulary VI procedure. It also increased the amount of tannin present in the first 500 ml. of fluidextract. Increasing the glycerin content of menstruum I also increased the amount of tannin found in the finished product, but the preparation was somewhat more viscous than the official fluidextract. The substitution of 8 parts of water and 2 parts of alcohol in place of the official menstruum I yielded less tannin. A heavy precipitate also occurred in these fluidextracts, indicating that highly aqueous menstruums are undesirable for the extraction of krameria.

The Use of Cylindrical and Conical Percolators in the Preparation of Fluidextract of Krameria.—Many workers, including Rosenwasser (5), Kelly and Krantz (6) and Breddin (7), have expressed the belief that long narrow percolators are to be preferred over broad conical percolators in the preparation of fluidextracts. Husa and Huyck (8) compared the efficiency of the Oldberg percolator, funnel, and tube of uniform diameter and found that the Oldberg percolator and the funnel gave better yields of alkaloids, whereas uniform glass tubes were somewhat more efficient for total extractive. The same workers later (9), working with a modified repetition diacolation process, found that the use of cylindrical tubes apparently had no advantages over the Oldberg percolators.

Three groups of fluidextracts were prepared from a number 20 powdered krameria root, one group in cylindrical tubes of uniform diameter, another in conical percolators and the third in cylindrical percolators, but omitting the collection and evaporation of weak percolates. The dimensions of the percolators were as follows:

1.	Cylindrical (uniform diameter)	
	Length of tube	150 cm.
	Inside diameter	49 mm.
	Outside diameter	51 mm.
	Length of drug column	80 cm.
	Ratio, diameter: length drug column	1:16
2.	Conical (slightly tapering)	
	Length	37 cm.
	Diameter at top	12.5 cm.
	Diameter at base	8.0 cm.
	Diameter at top of drug column	11.5 cm.
	Length of drug column	22.0 cm.
	Ratio, diameter: length drug column	1:2

The fluidextracts were assayed for tannin content and total extractive directly after completion. The results of the assays are shown in Table II.

Table II.—Results of Tannin and Total Extractive Assays

No.	Type of Percolator	Method of Extraction	Per Cent Tannin	Per Cent Total Extractive
A_1	Conical	N. F. VI	12.10	26.02
A_2	Conical	N. F. VI	12.41	26.07
A_3	Conical	N. F. VI	12.98	26.04
B_1	Cylindrical	N. F. VI	14.15	29.88
B_2	Cylindrical	N. F. VI	13.20	28.70
B_3	Cylindrical	N. F. VI	13.90	28.72
C_1	Cylindrical	Modified[a]	9.77	20.38
C_2	Cylindrical	Modified[a]	9.78	20.26
C_3	Cylindrical	Modified[a]	10.25	20.72

[a] Prepared without the collection and evaporation of weak percolates.

Comments: The results indicate that it is possible to extract somewhat more of the tannin from krameria if long cylindrical tubes are used rather than conical percolators. The fluidextracts of Group *C*, those prepared without the collection of weak percolates, showed that even with the advantage of a longer column of drug it was not possible to extract more than approximately 80% of the tannin obtained by the National Formulary VI method using conical percolators.

The Preparation of Fluidextract of Krameria by a Modified Diacolation Process.—The process of diacolation was devised by Breddin (10) and has undergone numerous modifications, including those made by the originator himself (11). This method of drug extraction has been investigated by Gstirner (12), Keller (13), Husa and Huyck (14) and others, most of whom have reported favorable results in its application.

The apparatus used in the experimental work of this study was similar to that of Breddin, as modified by Husa and Huyck (14). It consisted of a series of seven Pyrex glass tubes of 22 mm. outside diameter and 70 cm. length, connected by Pyrex glass U-tubes of the same diameter. The connections between straight sections and U-tubes were made with short lengths of three-quarter inch rubber tubing, three-quarter inch garden-hose clamps serving to tighten all such connections. Menstruum, contained in an air-tight tank, was forced through the drug, contained in the seven tubes, by means of compressed air obtained from the laboratory air jets. A fairly constant pressure of 9–13 pounds per square inch was available at all times.

Details concerning the apparatus are as follows:

Inside diameter of tubes	19 mm.
Outside diameter of tubes	22 mm.
Length of straight tubes	70 cm.
Length of U-tubes	22–24 cm.
Total length of drug column	700 cm.
Volume of packed drug	1915 ml.
Ratio, inside diameter:length drug column	1:368

Fluidextract of Krameria was prepared, in duplicate, by the modified diacolation process from 1000 Gm. of a number 20 powdered krameria root, 1000 ml. of finished fluidextract was collected, as were five additional fractions of 200 ml. each of the weak percolate. The various fractions of each fluidextract were assayed for tannin content and total extractive directly after they were collected. The results of the assays are shown in Tables III and IV.

Table III.—Results of Tannin Assays of Fractions of Percolate

Fraction	Per Cent Tannin Extracted Trial 1	Trial 2
First 1000 ml.	12.81	12.14
Weak percolates:		
First 200 ml.	0.79	0.78
Second 200 ml.	0.25	0.43
Third 200 ml.	0.16	0.43
Fourth 200 ml.	0.0	0.26
Fifth 200 ml.	0.0	0.0
Total	14.01	14.04

Table IV.—Results of Total Extractive Assays of Fractions of Percolate

Fraction	Per Cent Total Extractive Trial 1	Trial 2
First 1000 ml.	38.26	34.62
Weak percolates:		
First 200 ml.	2.87	4.24
Second 200 ml.	1.73	2.24
Third 200 ml.	1.17	1.67
Fourth 200 ml.	0.82	1.18
Fifth 200 ml.	0.88	0.92
Total	45.73	44.87

Comments: Most of the tannin was present in the first 1000 ml. of percolate. The small amount remaining in the drug was removed in subsequent weak percolates. The amount of tannin extracted

Table V.—Comparable Results of Different Methods of Extraction

No.	Percolator	Process	Per Cent Tannin Extracted	Per Cent Tannin Available	Efficiency[a]
A_1	Conical	N. F. VI	12.10	14.01[b]	86.3%
A_2	Conical	N. F. VI	11.40	14.01	81.4%
B_1	Cylinder	N. F. VI	13.05	14.01	93.0%
B_2	Cylinder	N. F. VI	12.95	14.01	92.4%
C_1	Cylinder	Modified	10.12	14.01	72.2%
C_2	Cylinder	Modified	10.09	14.01	72.0%
D_1	Diacolation		12.81	14.01	91.9%
D_2	Diacolation		12.14	14.01	86.9%

[a] A process which yielded a fluidextract containing 14.01% tannin would be 100% efficient.
[b] This figure was obtained from Table III and represents the amount of tannin present in the sample of krameria from which these products were prepared.

in the first 1000 ml. of percolate represented, in trial 1, 91.9% and in trial 2, 86.9% of all the tannin available in the drug. Variations in the two trials may be attributed to differences in time required for the menstruum to pass through the drug, probably caused by different pressures being exerted in packing the drug in the tubes.

Modified Diacolation Compared.—Fluidextracts of Krameria were prepared in conical and cylindrical percolators, the dimensions of which have already been outlined, by the National Formulary VI procedure, and by a modified process in which no weak percolates were collected. These were assayed and compared with the fluidextracts made by modified diacolation. The results are shown in Table V.

Comments: The results would indicate that modified diacolation has no advantage over the official process in which a long cylindrical tube is used as the percolator except, perhaps, economy of alcohol. The process does show advantages, however, over the National Formulary VI process conducted in a conical percolator.

Loss of Tannin in Fluidextract of Krameria.—All of the fluidextracts prepared in this study were assayed for tannin after they had aged for known periods of time. It was found that, in general, the amount of tannin lost in Fluidextracts of Krameria became greater with increased aging time. Although the fluidextracts prepared by modified diacolation were not allowed to age as long as the others, results showed that they exhibited approximately the same degree of instability.

SUMMARY AND CONCLUSIONS

1. It is not possible to extract the tannin from krameria completely and efficiently by ordinary percolation methods without the collection of weak percolates.

2. Increasing the length of the drug column seems to increase the efficiency of extraction when the National Formulary VI process of percolation is employed.

3. It is possible to remove approximately 90% of the tannin from krameria without the collection and evaporation of weak percolates by employing a modified diacolation procedure.

4. Modified diacolation seems to be preferable to the National Formulary VI process in which a conical percolator is employed for the preparation of Fluidextract of Krameria, but it apparently possesses no advantages over the same process using a long cylindrical tube as the percolator.

5. Losses of tannin in Fluidextracts of Krameria were studied. As high as 12.5% of the original tannin was lost over a period of four and one-half months.

6. As far as they were observed, Fluidextracts of Krameria prepared by a modified diacolation process lost their tannin at about the same rate as did those prepared by other methods.

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A Study of Hydrophile Ointment Bases*

By G. W. Johnston† and C. O. Lee‡

The so-called "Absorption Bases" are relatively new products. The word absorption is used to denote their hydrophile or water-holding property and not to describe their action when applied to the skin. However, claims are made for the absorption of many of these preparations which are being marketed under a variety of names.

These bases have found a definite place in pharmacy and cosmetology because of their special properties. Due to the interest and attention that they have been receiving it was felt that it would be worth while to attempt to produce a similar preparation from official substances.

* An abstract of a thesis presented to Purdue University in partial fulfilment of the requirements for the Master of Science degree by G. W. Johnston, August 1939.

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It is the aim of this paper to discuss the problem of making an absorption base comparable to the commercial products. Furthermore, it is our purpose to show that such a base may be used in the preparation of most of the ointments of the U. S. Pharmacopœia and the National Formulary.

WATER ABSORPTION BASES

Navarre (1) states that preparations of this kind are of two types: (*a*) those made with a concentrate dissolved in hydrocarbons, and (*b*) those made with a concentrate along with lanolin, hydrocarbons or lipids. It is further stated that absorption bases are compounds composed of the so-called oxycholesterol fraction of lanolin, referred to as the concentrate, fused with various hydrocarbon products such as petrolatum, mineral oil and paraffin, and with lanolin. The concentrates may contain 25 per cent or more of the oxycholesterols. It is claimed that the commercial absorption bases contain 5 to 10 per cent cholesterols in hydrocarbon bases.

The chief problem seems to be that of adding something to petrolatum to increase its water-holding property. Cetyl alcohol has been investigated in this respect. In 1917 Axelrod (2) presented a product with the following formula: petrolatum 70 parts, paraffin 20 parts, cetyl alcohol 10 parts, anhydrous wool fat 5 parts and water 100 parts.

Zugabe (3) reported an analysis of Eucerin, a trade-marked base. He claimed it to be composed of the following: cholesterol 5 per cent, anhydrous lanolin 15 per cent and petrolatum 80 per cent.

In the same year a formula was suggested for an anhydrous vaseline made from oxycholesterol 5 parts, wax and paraffin each 2 parts and liquid petrolatum 91 parts. It was claimed that this product was easily miscible with water (4).

Lifschuetz (5) discussed these bases and claimed that the water-absorbing properties of wool fat are attributable to its content of free alcohols, which are present to the extent of 5 to 8 per cent. It was also stated that upon the addition of 3 per cent of these alcohols to paraffin or vaseline, the latter acquired the property of absorbing a high content of water even up to 300 or 400 per cent.

In 1932 Schmatolla (6) wrote a series of articles upon increasing the hydrophilic properties of petrolatum. He presented formulas containing one-half to one per cent cholesterol in petrolatum bases. Seidler (7) at about the same time discussed the influence of cholesterol in ointments with a high water content.

Artl (8) in 1924 showed that the addition of one per cent of cetyl alcohol to petrolatum increased its water-absorbing power to 100 per cent. It has also been shown that the addition of 10 per cent of cetyl alcohol to lard will give a base capable of absorbing 100 per cent water.

In 1928 Wratschkoss (9) stated that he was able to prepare a water-absorbing base by heating petrolatum and glycerin to 280° in a thin-walled aluminum container. He did not mention the amount of water absorbed.

The method of Gottardo (10) is still different. He hydrogenated hydrocarbons and rendered them hydrophilic by means of the action of saturated solutions of salts, such as the chlorides of sodium, magnesium, calcium and iron. The properties of the hydrophilic petrolatums thus obtained varied with the salt used.

EXPERIMENTAL

The foregoing discussion of water-absorption bases, while brief, gives a clue to their approximate composition. The information to be found in the literature did not settle the problems of procedure in preparing such products. The matter of grades or quality of hydrocarbons, especially petrolatums, also waxes, wool fat and other constituents seemed to us to constitute sources of trouble.

We prepared 44 different bases using varying proportions of the following substances: cholesterol, white petrolatum, anhydrous lanolin, white wax, paraffin, ceresin, cetyl alcohol, oleic acid, yellow wax, liquid petrolatum, lecithin, cetaceum, glyceryl monostearate, sodium oleate, glycerin, sodium lauryl sulfonate, cholesterol esters, oxycholesterol and water.

It is not necessary to discuss all of these formulas in detail. The one which we found to be most likely in all respects was number 37. Its composition is as follows.

Cholesterol	5.0 Gm.
Anhydrous wool fat	20.0 "
Liquid petrolatum	45.0 "
Cetaceum	25.0 "
White wax	5.0 "

Procedure.—The wax and cetaceum are melted in a porcelain dish upon a water-bath. The lower melting point fats are then added and stirred sufficiently to insure a homogeneous mixture. The temperature is then increased to about 150° and the cholesterol added. Stirring is continued until solution is effected, after which the base is allowed to cool.

To incorporate the water the base is melted and the temperature maintained at about 45° to 50°. Water having the same temperature is then added in small portions with stirring until emulsification takes place, as evidenced by the formation of a white cream.

The limits of water absorption were determined for each of our formulas by repeated experimentation, the aim being to produce a base that would take up three or more times its weight of water.

Emulsification and Stability.—We were able to incorporate about 300 per cent of water in a base composed of 5 per cent each of lanolin and cholesterin in petrolatum early in our study but it was observed that upon standing or being rubbed upon the skin there was separation of water. An additional binding agent seemed necessary to produce more stable emulsification. Accordingly several such agents were tried, namely, cetyl alcohol, ceresin, lecithin, cetaceum, oleic acid, glyceryl monostearate, sodium lauryl sulfonate, white and yellow wax, etc. Of these agents white and yellow wax and cetaceum seemed to be the proper agents to add. These are to be found in our formula 37.

It was further observed that petrolatums varied greatly in their physical properties. This led us to abandon petrolatums in favor of the use of liquid petrolatum and relatively high percentages of white wax, cetaceum and lanolin.

Several investigators suggested the value of oxycholesterol and cholesterol esters in formulas of this kind. Our experience indicated that these substances do yield good water-holding formulas but that they lose their water after a week or so unless a rather large percentage of anhydrous lanolin is present. Oxycholesterol yielded products that were rather dark in color and which did not seem to exhibit as good emulsifying powers as those prepared with cholesterol.

Properties of Water-Holding Bases.—After a formula, satisfactory with respect to the emulsification of water is concerned, has been found there are many other factors to be considered. Among these are viscosity, melting point, spreading quality, adhesiveness and texture.

In general appearance and behavior, our base 37 and some of those on the market were quite alike. However, they were not alike in all physical respects as shown in Table I.

These tests were carried out according to the directions of the U. S. Pharmacopœia XI.

Pharmaceutical Application of Absorption Bases.—After formulating base 37, it was decided to study the limitations of its use in making ointments of official medicinal strength. Aquaphor was used as the control base.

The general procedure was as follows: The proper amounts of the base and water were formed into an emulsion cream. The medicinal ingredients were then levigated with a portion of the emulsion until smooth, and the remainder gradually incorporated. In the case of liquid ingredients simple admixture with the cream was all that was needed. Where solids and liquids were both present they were mixed, then added to the cream as described above.

We were able to make very satisfactory U. S. Pharmacopœia ointments of: boric acid, tannic acid, rose water, chrysarobin, ammoniated mercury, yellow mercuric oxide, iodine and zinc oxide. Ointment of pine tar was too soft and the addition of 10 per cent of white wax was necessary. Ointments of belladonna, nutgall and phenol were unsatisfactory in that they caused the emulsion cream to break. Doubtless this difficulty can be overcome with further study.

Of the National Formulary ointments the following proved to be satisfactory in appearance and general properties: compound ointment of benzoic acid, calamine, capsicum, mild mercurous chloride, mercuric nitrate, red mercuric oxide, stainless iodized ointment, coal tar, compound ointment of tar, lead oleate, potassium iodide, scarlet red, mustard, alkaline sulfur ointment and zinc stearate.

Ointment of ichthammol was too soft and required the addition of white wax equal to the amount of ichthammol. We were unable to produce a satisfactory ointment of menthol or resorcinol.

Stability of Absorption Base Ointments.—The official ointments, as described, prepared with absorption bases were subjected to variable temperatures in order to determine their stability at summer heat and at lower temperatures. They were placed in an oven at 33° for 24 hours. Separate samples were placed in a refrigerator for the same length of time at 10°.

Of the 25 ointments studied about 10% of them were stable at 33° but only about 40% of them showed stability at 10°. Those which showed separation of any kind under this treatment were considered unstable.

The water content of these ointments was variable due to their variations in medicinal agents, and the base. In all instances, 25 Gm. of the base were used, with sufficient active ingredients for a 100-Gm.

Table I.—Constants of Ointment Bases

Base	Engler Degrees	Kinematic Viscosity	Melting Point
Aquaphor	1.68	0.080	40.0°
Base 37	2.21	0.135	41.1°

formula of official strength ointment, and enough water to make 100 Gm. of the product. The water content ranged from 100 per cent to 300 per cent. About 50 per cent of these showed no loss of water over a period of 25 days at room temperature even during summer heat. Several showed loss of water within a few days.

CONCLUSIONS

These observations have led us to conclude that ointments prepared with water-absorption bases containing fairly high amounts of water are sensitive to temperature changes. For this reason we believe that hydrophile ointment bases are much less suited to stock ointments than to those for extemporaneous use.

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Book Reviews

The Squibb Ancient Pharmacy, by GEORGE URDANG and F. W. NITARDY. 190 pages, 4¼ x 7. E. R. Squibb & Sons, New York, 1940. Price, $1.00.

This little volume is a catalog of a European collection of old drug jars, mortars, utensils, furnishings and apothecary shop fixtures, acquired by E. R. Squibb & Sons of New York in 1932. This collection is housed in two rooms on the 28th floor of the Squibb Building at 58th Street and Fifth Avenue, New York. The rooms are designed in the style of an old apothecary shop and are open to inspection by pharmacists and other interested persons. The catalog gives detailed descriptions of the old faience ware, glassware, mortars, pictures, books and documents. The text is well illustrated and contains many historical data which should be of interest to all pharmacists who take pride in their calling.—A. G. D.

The Homeopathic Pharmacopœia of the United States. Published under the direction of the Committee on Pharmacopœia of the American Institute of Homeopathy. Fifth Edition, revised, price, $6.00. First Supplement, 50¢. The publications may be purchased from Boericke & Tafel, Homeopathic Pharmacists.

The Homeopathic Pharmacopœia is one of the books on drug standards named in the Federal Food, Drug and Cosmetic Act. It deals with the drugs and medicines used by the homeopathic practitioners and is prepared by the Committee on Pharmacopœia of the American Institute of Homeopathy and collaborators. The present members of the committee are T. H. Carmichael, M.D., Chairman; G. W. Boericke, M.D.; H. S. Nicholson, M.D.; J. S. Stewart, M.D.; F. F. Massey, M.D. In addition to listing standards for drugs and medicines used in homeopathic practice, the volume also gives information relative to their botany, chemistry, pharmacy and pharmacology, also the preparation of dilutions and dosages. Pharmacists who are called upon to fill homeopathic prescriptions should provide themselves with the revised edition of this Pharmacopœia as homeopathic preparations must conform to the standards provided thereby.—E. G. E.

A reprint of *German Medicinal Plants, No. 8, 1938*. A contribution to the knowledge of the drug, *Herba Lobelia*, and requirements for its cultivation by DR. ISE ESDORN, from the Institute of Applied Botany, Hamburg.

The method of cultivation of lobelia is described—the flowering time in Germany is July to August and the harvesting season is near the end of the flowering time. The author states that the cultivation presents no great difficulties and the market price of the drug is satisfactory. The roots and seeds are described, also methods of analysis. The alkaloidal strength of the dried drug decreases some on storage—it dropped from 0.36% to 0.34% in six months.—E. G. E.

Argyria—the Pharmacology of Silver. By WILLIAM R. HILL, M.D., Instructor in Dermatology and Syphilology, University of Pennsylvania, and DONALD M. PILLSBURG, M.A., Associate Professor of Dermatology and Syphilology. Published November 1939 by Williams and Wilkins Company, Baltimore. Price, $2.50, bound in cloth.

"The study of the pharmacology of silver in relation to the problem of argyria has been carried out

at the instance of Lawrence Addicks for the American Silver Producers' Research Project under a Fellowship established in the Department of Dermatology and Syphilology of the University of Pennsylvania"—quoted from the preface. The preface states the primary purpose to be a review of the literature of the subject in an attempt to formulate conclusions concerning the incidence of argyria. The source material has been studied and, with few exceptions, the authors have been able to consult the original papers. The subject matter is presented in nine chapters of more than 160 pages. Chapter I is devoted to history of silver pharmacology. The term "argyria" according to the authors was, probably, first employed by C. H. Fuchs, Göttingen, and "argyrosis," indicating pigmentation of the eye by silver, was first investigated by E. Junge (*Graefe's Arch.*, 5 (1857), 197). The bibliography contains six hundred titles; the authors' index has about the same number of titles and there is a corresponding subject index; a list of nearly one hundred names is required for the Proprietary Silver Compounds, and a bibliography of seventeen references. All of these are helpful for library research and other studies on the subject. The chapters on absorption, transportation and excretion of silver; the deposition of silver in the tissues; pharmaco-physiologic effects of silver administration; diagnosis and treatment of argyria; analysis of reported cases of argyria; production and prevention of argyria; argyria of the eye (argyrosis); and industrial argyria complete the interesting discussions.—E. G. E.

Clinical Toxicology, by CLINTON H. THIENES, M.D., Ph.D., Professor of Pharmacology, School of Medicine, University of Southern California, Los Angeles; Attending Pathologist (Toxicology), Los Angeles County Hospital. Flexible binding, 309 pages, $5^1/_4$ x 8. Philadelphia: Lea & Febiger, 1940. Price, $3.50.

This concise little treatise is intended as a classroom textbook and as a guide for the practicing physician and toxicologist. Only such chemistry, pharmacology and pharmacy are given as are required for the understanding of the toxicology of the materials considered. Full descriptions of poisons are limited to those which most frequently give rise to distressing symptoms or death and to those which serve to typify a group of poisons. Poisons are classified according to their major toxic actions rather than to their positions in the alphabet or their chemical classifications. This is done in order to facilitate diagnosis and treatment. Where a poison has an action on more than one physiological system, it is mentioned under more than one heading. Chemical tests which give the best reactions for identification are described and some biological tests are also included. The treatment of cases of poisoning is described from the point of view of pharmacology and pathology. There are nine sections in the book. The first six are devoted to the toxicology of the following classes of poisons: convulsant poisons, central nervous system depressants, peripherally acting nerve poisons, muscle poisons, protoplasmic poisons and poisons of the blood and hematopoietic organs. The remaining three sections deal with treatment and include an outline of symptom diagnosis and chemical diagnosis of poisoning.—A. G. D.

The Association has received a dissertation from the author on *Fluoreszen Mikroskopische Untersuchungen an Rindenpulvern unter Zuhilfsnahme von Reagenzien* (Fluorescent Microscopical Investigation of Powdered Barks with the Aid of Reagents)—a thesis submitted in partial fulfilment of the requirements for the Ph.D. degree at the University of Basel by IRMA STEINER, Apothecary, fem., in Canton Solothurn, Switzerland. Publisher Julius Henburger, Basel; dedicated to the author's parents.

In the special part of the work which follows the Introduction and the General Discussions, the characteristics of twenty-five powdered barks are described in detail. Using the first bark of the list, Angostura bark, *e. g.*, the different structures comprising the bark are described and the color reactions with reagents are defined. The latter do not differ greatly from those described in the Bulletins of the National Formulary which have been carefully systematized and to which reference can be made. The author concludes her work by listing the drugs of her research and has prepared a useful table in which the reagents are tabulated and also the color designations.—E. G. E.

Text Book of Pharmacognosy, by GEORGE EDWARD TREASE. 3rd ed., x + 739 pages, $5^1/_2$ x $8^1/_2$. Baltimore: Williams & Wilkins, 1939. Price, $6.00.

This is the third edition of the well-known text by this author, who is lecturer on pharmacognosy in the University College of Nottingham, England. The first chapters are devoted to a historical account of the commerce in drugs and include a description of the drug auctions conducted under the Port of London Authority. There are also chapters on the collection and cultivation of drugs, insects attacking drugs, plant principles and enzymes. Part II deals with the structure of plants and the microscopy of drugs. Part III contains a classification of vegetable drugs according to their taxonomic position. Part IV deals with drugs of animal origin and contains a summary of the various methods of drug analysis. The book is well illustrated and the format is good. It differs from the other books in this field in that greater attention is given to the commerce in drugs, such features as packing, grades and prices being discussed. The book should be in the library of every pharmacist.—A. G. D.